Title of the project

Bone Properties and Biomechanics in Patients with Psoriatic Disease: A Prospective Study with High-Resolution Peripheral Quantitative Computed Tomography (HRpQCT)

Scientific abstract

Psoriatic disease has unique and complex effects on bone metabolism, resulting in both pathological bone formation and bone resorption [1]. IL-17, TNF and Wnt system are crucial in fine-tuning the pathogenesis of bone changes of psoriatic diseases [2–4]. The study's primary objective is to unveil the effects of IL-17 inhibition and TNF inhibition on bone properties and biomechanics in patients with psoriatic disease. We will explore, longitudinally, the changes in HRpQCT parameters at the distal radius in patients with either psoriasis or psoriatic arthritis who receive anti-IL-17 or anti-TNF drugs. We will also assess structural modifications of distal interphalangeal joints (DIP), interphalangeal joints (PIP), and metacarpophalangeal joints (MCP) in response to such drugs. In exploratory analyses, we will test the differential effects of IL-17 inhibition and TNF inhibition on standard DXA, radiofrequency echographic multispectrometry (REMS), serum markers, and serum miRNA expression related to bone metabolism (e.g., Wnt system and its inhibitors Dkk1 and sclerostin, bone turnover markers etc.). We plan to enroll 40 patients in the study and follow them for up to 12 months, with visits scheduled every 3 months. Including patients with psoriatic arthritis and psoriasis without arthritis is crucial. Including both populations will allow us to isolate the specific effects of systemic inflammation on bone health. By comparing these two groups, we aim to distinguish how joint inflammation in psoriatic arthritis contributes to bone changes, separate from the skin inflammation seen in psoriasis. This differentiation is crucial for understanding the underlying mechanisms of bone metabolism in psoriatic disease.

Lay abstract

Psoriatic disease can lead to unusual bone growth and an increased risk of bone loss or fractures. Our study aims to explore how certain treatments, specifically those targeting inflammation through IL-17 and TNF inhibitors, affect bone structure and strength in people with psoriatic disease. Using a cutting-edge imaging technique, HRpQCT, we will closely examine the bones of the hands and wrists of patients undergoing these treatments. This method gives us a detailed three-dimensional view of the bone, allowing us to see changes in bone density and structure that other methods might miss. By observing patients over a year, we hope to understand how these treatments impact bone formation and loss. We will also look at how the treatments might affect the joints most commonly involved in psoriatic disease, such as those in the fingers and hands, by looking for signs of erosion or bone growth. In addition to imaging, we will study blood markers and miRNA (small molecules that can influence gene expression) related to bone health. This study aims to provide new insights into the bone effects of psoriatic disease.

Background

Psoriatic arthritis has been associated with an increased risk of fractures [5–7], which is at least in part attributed to the prevalent inflammation. However, even cutaneous psoriasis seemed to confer a similar risk of osteoporosis and fractures [7]. Psoriatic disease is indeed characterized by a complex pathophysiology; on the osteometabolic side it involves both bone erosion and bone apposition. The Wnt pathway, known for its pivotal role in bone formation and remodeling, could have a multifaceted influence on these processes [8–10]. In recent years has become more apparent that the Wnt system is modulated by inflammatory cytokines such as IL-17 and TNF [11–14]. Such cytokines can, for example, suppress the Wnt system, enhancing osteoclastogenesis and leading to increased bone resorption. Conversely, in certain microenvironments within the joint, the Wnt pathway could be activated by IL17 [15,16], leading to abnormal bone apposition. This dual action of the Wnt system in response to different cytokines could explain the phenomenon of bone apposition seen in psoriatic disease, such as the development of enthesophytes. This is a particularly intriguing area of speculation, especially when considering the interplay between the Wnt system, IL-17 and TNF. The collaboration between the rheumatology and dermatology sections at the University of Verona has led to a series of pilot studies that testify the strong partnership between units [13,14,17,17–22]. HRpQCT represents a state-of-the-art imaging modality that offers a remarkable three-dimensional assessment of bone quality and architecture, surpassing the capabilities of traditional imaging techniques in the evaluation of bone health, especially within the context of inflammatory arthritis. HRpQCT has been used to assess bone health in psoriatic arthritis [23,24], however a comparative, in-depth, analysis of psoriasis and psoriatic

arthritis, including miRNA and markers analysis, is novel. In addition, no study compared the effect of IL17 inhibition on such parameters.

Methods

We will enroll 20 patients affected by psoriatic arthritis and 20 patients with isolated cutaneous psoriasis in this prospective observational study.

Inclusion criteria

- ≥18 years of age
- Diagnosis of psoriasis (n=20) or diagnosis of psoriatic arthritis (n=20) (the latter satisfying CASPAR criteria)
- Psoriatic arthritis with peripheral arthritis involving hands
- Naïve to any biological DMARD or targeted synthetic DMARD, conventional synthetic DMARD will be allowed.
- Initiating an anti-TNF (n=20) or an anti-IL17 (n=20), decision upon clinical judgment of the treating dermatologist or rheumatologist, to ensure comparability we will try to include patients with similar comorbidities and characteristics, however we will not randomized patients

Exclusion criteria

- Past treatment with any anti-osteoporosis medication in the past 5 years (including bisphosphonates, PTH analogs, romosozumab and denosumab), 10 years for zoledronic acid.
- Unstable systemic medical condition
- History of hypo- or hyperparathyroidism, osteomalacia, Paget's disease of bone or other bone diseases which affect bone metabolism.
- Abnormalities of the following per central laboratory reference ranges: Vitamin D deficiency [25(OH) vitamin D level <20 ng/mL (<49.9 nmol/L)] and hypercalcemia

Study procedures

HRpQCT will be performed at the non-dominant hand at baseline, month 6 and month 12

We will use the ARTiCAT HRpQCT scanner by RAR Srl. following the manufacturer's standard in vivo imaging protocol. ARTiCAT uses as its X-ray source a monoblock device with 50 W of power operating at 80 kV and with a microfocus radiogenic tube (nominal 33 micrometers) and as a detector a CMOS-type 'flat-panel', with dimensions 153 x 153 mm. ARTiCAT LargeField HR protocol have a ROI of 4.5 cm x 4.5 cm and voxel size of 65 μm (see examples of images and video below).

- Distal Radius: Reference line on the positioning, typically 9.5-15 mm from the joint space (radio-carpal). Bone microstructure will be evaluated at the distal radius with the following parameters: Trabecular vBMD, mg HA/cm3 (primary outcome) Total vBMD, mg HA/cm3 Cortical vBMD, mg HA/cm3 Trabecular bone volume fraction (BV/TV) Trabecular thickness (Tb.Th) Trabecular separation (Tb.Sp) Trabecular number (Tb.N) Cortical thickness (Ct.Th) Cortical porosity Micro-finite element analysis (uFEA).
- DIP, PIP and MCP: Set the scanning region to cover 10 mm above and below the joint space of the II and III DIP, PIP and MCF. Joints will be evaluated for erosions (number and volume) by an experienced rheumatologist. Erosion volume (mm3) will be measured manually on the largest erosion. Bone neoapposition (enthesophyte) will also be manually measured at the joint site following standardized procedures [25].

DXA will be performed at baseline, month 6 and month 12

Standard DXA at lumbar spine, distal radius, total-hip and femoral neck + TBS analysis with iDXA Lunar (General Electric) will be conducted.

REMS will be performed at baseline, month 6 and month 12

REMS scans of lumbar vertebrae and proximal femur were performed employing a dedicated echographic device (EchoStation, Echolight Spa, Lecce, Italy), equipped with a convex transducer operating at the nominal frequency of 3.5 MHz and used as recommended by the manufacturer

Serum markers and miRNA will be performed at baseline, month 6 and month 12

PBMCs will be freshly isolated using density gradient centrifugation from heparinized peripheral blood. PBMCs will be stored at −80°C in freezing medium for later use. Low-molecular weight RNAs of less than 200 nt will be extracted from the PBMCs. RNA will be purified from PBMC using the miRCURY RNA Isolation Kit (Exiqon) according to the manufacturer's instructions.

High throughput and single miRNA expression will be analysed using the TaqMan® Human microRNA Array (Card Set v3.0, Applied Biosystems) and the TaqMan® microRNA Human Assays (Applied Biosystems), respectively, on Real-time PCR QuantStudio3. Expression levels of microRNAs will be normalised to the expression level of small nuclear RNA U6 (U6 snRNA) as internal control. Examples of miRNA to be studied (related to bone metabolism): DKK1: hsa-miR-217-5p, SOST: hsa-miR-411-3p, WNT3A: hsa-miR-1915- 3p, etc. (list is not complete. >100 miRNA). In order to select miRNA candidates, we have accessed the mirDIP v4.1 database, providing nearly 152 million human microRNA–target predictions, which were collected across 30 different resources. mirDIP v4.1 is freely available at http://ophid.utoronto.ca/mirDIP/. More than 100 miRNA will be cautiously selected. Several bone markers (P1nP, CTX, Dkk1, Sclerostin, Periostin, PTH, vitamin D etc.) will be analyzed with IDS-ISYS Multi-Discipline Automated Analyzer based on chemiluminescence technology.

Sample size and statistical considerations

Given the study's exploratory nature, we could not provide a solid sample size justification. Sample size has been estimated for primary outcome trabecular vBMD measured with HRpQCT. A previous systematic review indicated a 14.9% (95% CI 6.9-22.9) difference in vBMD measured with HRpQCT between psoriatic disease patients and controls. We presume a beneficial effect of bDMARDs on bone properties possibly restoring 50% of vBMD (+7.5%) maintaining a similar standard deviation.

Comparison of changes in HRpQCT trabecular vBMD across the study groups will be analyzed using General Linear Model (GLM) or mixed-effects model for repeated measures with the within-subject factor is time (baseline and 12 months). The between subjects' factor is the treatment scheme.

Generally, in microarray experiments the aim is to find miRNA or proteins that are differentially expressed between two samples. We are planning a study of a continuous response variable from matched pairs of study subjects, eventually we will try to detect differentially expressed genes (prior and post therapy) by performing separate gene-by-gene (or protein-by-protein) t-tests. The sample size depends on how large a difference you want to be able to detect (typically measured as the fold difference between the samples). For sample size purposes we also need the standard deviation of the miRNA intensity measurements on the base-two logarithmic scale. A standard deviation of 0.7 is fairly realistic for miRNA that are expressed at moderate to high levels [26]. We expect at least a 1.5-fold change from baseline to month 6 and we will study approximately 250 genes/proteins in study. Accepting 2 false positive tests and with a power of 0.8 we will need to study 20 pairs of samples. We will also execute a series of multiple linear regression models with log-normalized protein/miRNA expression as the dependent variable and HRpQCT parameters as the independent variable. We will apply the Benjamini–Hochberg procedure for controlling the False Discovery Rate (FDR) to all p-values with an a priori p-value of 0.05 for identifying statistically significant proteins in all models.

Expected results

In this study, we anticipate discovering crucial insights into the differential impacts of IL-17 and TNF inhibitors on bone health among patients with psoriatic disease. We specifically expect that blocking IL-17 will more effectively stimulate bone formation than TNF inhibition. Through detailed HRpQCT imaging, we aim to document the variations in bone microstructure and density, demonstrating the potential superiority of IL-17 inhibitors in counteracting the adverse effects of psoriatic disease on bones. Additionally, we predict that alterations in serum markers and miRNA expressions, which are closely linked to bone metabolism, will further substantiate the distinct biological impacts of these therapies. These outcomes could highlight the importance of targeted therapeutic choices in preserving bone integrity and preventing osteoporosis in patients afflicted with psoriatic disease.

Significance for psoriatic disease

This research holds considerable significance for treating and managing psoriatic disease by potentially confirming the superior role of IL-17 blockade in promoting bone health. By revealing the specific effects of IL-17 and TNF inhibitors on bone metabolism, the study could pave the way for more personalized treatment strategies that not only address the skin and joint symptoms of psoriatic disease but also proactively enhance bone formation and reduce the risk of bone loss. Understanding these dynamics could empower healthcare providers to optimize therapeutic interventions, ultimately improving the long-term musculoskeletal health and quality of life for individuals with psoriatic disease.

References

1 Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. *N Engl J Med*. 2017;376:957–70.
2 Rossini M, Viapiana O, Adami S, *et al.* Focal bone involvement in inflammatory arthritis: the role of IL17. *Rheumatology International*. 2016;36:469–82.
3 Adami S, Cavani A, Rossi F, *et al.* The role of interleukin-17A in psoriatic disease. *BioDrugs*. 2014;28:487–97.
4 Adami G. Regulation of bone mass in inflammatory diseases. *Best Pract Res Clin Endocrinol Metab*. 2021;101611.
5 Chen T-L, Lu J-W, Huang Y-W, *et al.* Bone Mineral Density, Osteoporosis, and Fracture Risk in Adult Patients with Psoriasis or Psoriatic Arthritis: A Systematic Review and Meta-Analysis of Observational Studies. *J Clin Med*. 2020;9:3712.
6 Sepehri NZ, Raeisi T, Razi B, *et al.* The association between psoriasis and psoriatic arthritis with the risk of osteoporosis, osteopenia and bone fractures: A systematic review and meta-analysis. *Int J Clin Pract*. 2021;75:e14630.
7 Ogdie A, Harter L, Shin D, *et al.* The risk of fracture among patients with psoriatic arthritis and psoriasis: a population-based study. *Ann Rheum Dis*. 2017;76:882–5.
8 Rossini M, Gatti D, Adami S. Involvement of WNT/β-catenin signaling in the treatment of osteoporosis. *Calcif Tissue Int*. 2013;93:121–32.
9 Churchman SM, Ponchel F, Boxall SA, *et al.* Transcriptional profile of native CD271+ multipotential stromal cells: evidence for multiple fates, with prominent osteogenic and Wnt pathway signaling activity. *Arthritis Rheum*. 2012;64:2632–43.
10 Diarra D, Stolina M, Polzer K, *et al.* Dickkopf-1 is a master regulator of joint remodeling. *Nat Med*. 2007;13:156–63.
11 Adami G, Orsolini G, Adami S, *et al.* Effects of TNF Inhibitors on Parathyroid Hormone and Wnt Signaling Antagonists in Rheumatoid Arthritis. *Calcified Tissue International*. 2016;99:360–4.
12 Adami G, Fassio A, Rossini M, *et al.* Machine learning to characterize bone biomarkers profile in rheumatoid arthritis. *Front Immunol*. 2023;14:1291727.
13 Fassio A, Gatti D, Rossini M, *et al.* Secukinumab produces a quick increase in WNT signalling antagonists in patients with psoriatic arthritis. *Clin Exp Rheumatol*. 2019;37:133–6.
14 Fassio A, Gatti D, Gisondi P, *et al.* Effects of secukinumab on serum adipocytokines: Preliminary data. *Reumatismo*. 2017;69:105–10.
15 Russell T, Watad A, Bridgewood C, *et al.* IL-17A and TNF Modulate Normal Human Spinal Entheseal Bone and Soft Tissue Mesenchymal Stem Cell Osteogenesis, Adipogenesis, and Stromal Function. *Cells*. 2021;10:341.
16 Russell T, Bridgewood C, Rowe H, *et al.* Cytokine “fine tuning” of enthesis tissue homeostasis as a pointer to spondyloarthritis pathogenesis with a focus on relevant TNF and IL-17 targeted therapies. *Semin Immunopathol*. 2021;43:193–206.
17 Gisondi P, Rossini M, Di Cesare A, *et al.* Vitamin D status in patients with chronic plaque psoriasis. *British Journal of Dermatology*. 2012;166:505–10.
18 Bellinato F, Adami G, Vaienti S, *et al.* Association Between Short-term Exposure to Environmental Air Pollution and Psoriasis Flare. JAMA Dermatology. 2022;158:375–81. https://doi.org/10.1001/jamadermatol.2021.6019
19 Bellinato F, Gisondi P, Filippucci E, *et al.* Systematic study on nail plate assessment: differences in nail plate shape, thickness, power Doppler signal and scanning approach. Archives of Dermatological Research. 2023;315:593–600. https://doi.org/10.1007/s00403-022-02404-5
20 Bellinato F, Adami G, Furci A, *et al.* Association between short-term exposure to environmental air pollution and atopic dermatitis flare in patients treated with dupilumab. JAAD International. 2023;11:72–7. https://doi.org/10.1016/j.jdin.2023.01.018
21 Idolazzi L, Zabotti A, Fassio A, *et al.* The ultrasonographic study of the nail reveals differences in patients affected by inflammatory and degenerative conditions. *Clinical Rheumatology*. 2019;38:913–20.
22 Gisondi P, Bellinato F, Targher G, *et al.* Biological disease-modifying antirheumatic drugs may mitigate the risk of psoriatic arthritis in patients with chronic plaque psoriasis. *Ann Rheum Dis*. 2022;81:68–73.

23 Wu D, Griffith JF, Lam SHM, *et al.* Progressive structural bone changes and their relationship with treatment in patients with psoriatic arthritis: a longitudinal HR-pQCT study. *Arthritis Res Ther*. 2019;21:265.
24 Qi F, Yao A, He Y. Medical imaging examination in psoriasis and early psoriatic arthritis patients: an updated systematic review and meta-analysis. *Int J Dermatol*. 2021;60:1354–62.
25 Moen HV, Hänel M, Therkildsen J, *et al.* Bony proliferations in rheumatoid arthritis, psoriatic arthritis and osteoarthritis using high-resolution peripheral quantitative computed tomography-A systematic literature review. *Int J Rheum Dis*. 2024;27:e15068.
26 Lin W-J, Hsueh H-M, Chen JJ. Power and sample size estimation in microarray studies. *BMC Bioinformatics*. 2010;11:48.

Video 1 - HRpQCT – Psoriatic arthritis patient's DIP

double click here:

double click here:

Image 1 - HRpQCT – Healthy control wrist

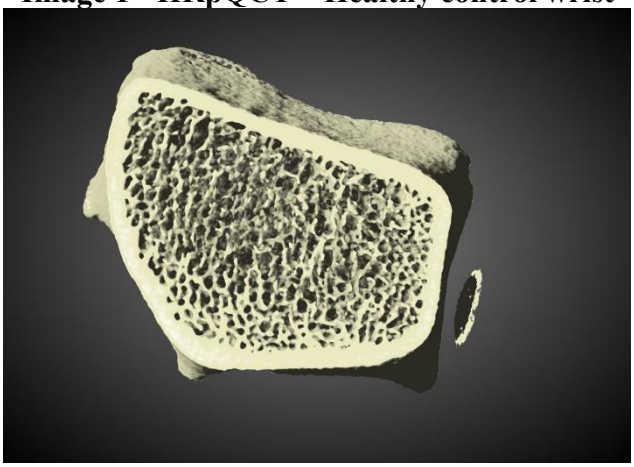

Image 2 - HRpQCT – Psoriatic arthritis patient's wrist