TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title
Unravelling Methotrexate osteopathy

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Background

Methotrexate (MTX) remains a cornerstone therapy for managing autoimmune diseases such as rheumatoid arthritis (RA), psoriatic arthritis, and connective tissue disorders like systemic lupus erythematosus (SLE). Recently, multiple case series have highlighted an emerging condition known as Methotrexate-associated insufficiency fractures, or Methotrexate osteopathy (MTXO). This condition is characterized by atraumatic fractures, bone pain, and distinctive radiological findings, including band- or meander-shaped stress fractures near growth plates. Due to the limitations of conventional radiographs in detecting insufficiency fractures, the average diagnostic delay exceeds six months. This underscores a significant, under-recognized burden on patients and highlights the need for further research. [1]Our research group recently demonstrated that continuing MTX in patients with MTXO-related insufficiency fractures significantly increases the risk of additional insufficiency and major osteoporotic fractures. Furthermore, fracture healing in MTXO cases is frequently delayed or incomplete, leading to chronic pain and long-term disability.[2]

The pathophysiology of MTXO remains poorly understood. While MTX-induced suppression of osteoblast activity was proposed as a mechanism, this effect has only been observed in animal studies (e.g., rats), and low-dose MTX-induced osteoblast suppression has not been validated in human studies. Moreover, the lack of evidence linking MTX to changes in bone mineral density (BMD) suggests a possible idiosyncratic reaction of bone cells or turnover mechanisms to MTX. [3]The precise effects of MTX on bone biology and fracture healing are still largely unexamined.

Epidemiological data on MTXO are limited due to diagnostic challenges, misdiagnosis, and under-reporting. Advanced imaging techniques such as MRI and CT are critical for detecting the subtle fracture patterns characteristic of MTXO. Hence, the prevalence of MTXO remains unknown. It is also unclear whether genetic factors may influence individual susceptibility to MTXO. Variations in genes linked to bone turnover, fractures or drug metabolism could predispose certain individuals to this condition. To investigate this, we would propose a candidate gene approach in MTXO patients and matched controls.

In addition to clinical and genetic factors, the effects of MTX on bone biology require further exploration. For example, zebrafish models have shown that MTX can cause craniofacial abnormalities, suggesting potential impacts on cartilage formation[4]. However, the effects of MTX on specific bone cells, such as osteocytes, and its role in fracture healing remain unknown. These models offer valuable insights into MTX's mechanistic impact on bone development, turnover, and repair in vivo.

This project aims to bridge these knowledge gaps by integrating population-level epidemiological studies, genetic investigations, and preclinical modelling. Through this approach, we hope to develop a

comprehensive understanding of MTX's effects on bone health and identify strategies to mitigate its adverse outcomes.

Aims

1. **Epidemiological Analysis:** Utilize electronic health record data from a national rheumatology registry to examine the association between MTX, other disease-modifying antirheumatic drugs (DMARDs), and the risk of insufficiency fractures and major osteoporotic fractures.
2. **Predictor Identification:** Investigate genetic and clinical predictors of MTXO through stratified patient analysis.
3. **Preclinical Insights:** Explore MTX's effects on bone turnover, development, and fracture healing using a zebrafish model, focusing on pathways such as osteoblast inhibition and cytokine-mediated bone resorption.

Training and experience provided

1) **Epidemiological Skills:** Training in advanced statistical analysis and data handling using large-scale electronic health records.
2) **Analysis of clinical and genetic risk factors:** Candidate gene association analysis, bioinformatics, and identification of biomarkers associated with MTX-related skeletal toxicity.
3) **Translational Research Techniques:** Practical training in zebrafish models, including micro-CT imaging, dynamic histomorphometry, and molecular analyses of bone-related genes and pathways.
4) **Collaborative Opportunities:** Exposure to interdisciplinary collaboration with rheumatologists and osteologists (group based in Hamburg), epidemiologists, and bone biologists, leveraging expertise from clinical and preclinical domains.

Expected outcomes

1. **Population-Level Insights:** Clarify the prevalence of insufficiency and osteoporotic fractures among users of MTX and other conventional or biologic DMARDs
2. **Clinical and Genetic Biomarker Identification:** Discover novel genetic markers predictive of MTXO, potentially enabling personalized medicine approaches for MTX therapy.
3. **Mechanistic Understanding:** Define MTX-induced changes in bone microarchitecture and fracture healing in vivo, providing mechanistic insights into MTXO pathogenesis and therapeutic avenues.
4. **Policy Impact:** Inform clinical guidelines on MTX monitoring and fracture prevention in high-risk populations, emphasizing early diagnosis and tailored treatment cessation strategies.

References

[1] Ruffer, N., Krusche, M., Beil, F. T., Amling, M., Kotter, I., & Rolvien, T. (2022). Clinical features of methotrexate osteopathy in rheumatic musculoskeletal disease: A systematic review. *Semin Arthritis Rheum*, *52*, 151952. https://doi.org/10.1016/j.semarthrit.2022.151952

[2] Hauser B, Merriman A, Foley J*, et al* OP0090 Continuos treatment with Methotrexate leads to increased overall fracture rate in patients who sustained Methotrexate associated lower limb insufficiency fractures *Annals of the Rheumatic Diseases* 2024;**83:**147-148

[3] Hauser B, Raterman H, Ralston SH, Lems WF. The Effect of Anti-rheumatic Drugs on the Skeleton. Calcif Tissue Int. 2022 Nov;111(5):445-456. doi: 10.1007/s00223-022-01001-y.

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