SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Effects of JAK inhibition on bone loss and vascular inflammation in rheumatoid arthritis

by Attila Hamar, MD

Supervisor: Szilvia Szamosi, MD, PhD

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2023

Effects of JAK inhibition on bone loss and vascular inflammation in rheumatoid arthritis

By Attila Hamar, MD

Supervisor: Szilvia Szamosi MD,PhD

Doctoral School of Clinical Medicine, University of Debrecen

Head of the **Examination Committee:**	Norbert Németh, MD, PhD, Dsc
Members of the Examination Committee:	Péter Antal-Szalmás, MD, PhD, Dsc
	Tamás Németh, MD, PhD

The examination takes place at the Library of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen,

22nd January, 2024, 11:00 am.

Head of the **Defense Committee:**	Norbert Németh, MD, PhD, Dsc
Reviewers:	Antónia Szántó, MD, PhD
	Attila Balog, MD, PhD
Members of the Defense Committee:	Péter Antal-Szalmás, MD, PhD, Dsc
	Tamás Németh, MD, PhD

The PhD Defense takes place at the Lecture Hall of Building. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen,

22nd January 2024, 13:00 pm.

1. Introduction, literature review

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory condition, resulting in symmetrical polyarthritis and joint damage leading to physical disability. Studies indicate that patients with RA have an increased susceptibility to cardiovascular disease (CVD), which is the primary cause of mortality in this patient population. Apart from the increased likelihood of CVD, individuals with RA are also susceptible to localized and systemic osteoporosis, which may lead to an increased risk of fragility fractures. The presence of chronic systemic inflammation associated with RA plays a significant role in the development of both bone loss and CVD. In the recent years, several new treatments have been introduced for the therapy of RA, with the latest of Janus kinase (JAK) inhibitors. These inhibitors are able to block the signaling pathway of various cytokines, hormones and growth factors, allowing the reduction of inflammation with a single synthetic compound. Inflammation is recognized as a contributing factor to the pathogenesis of atherosclerosis, and emerging evidence indicates that the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway also has a significant impact on bone metabolism and turnover.

1.1. Epidemiology and etiopathogenesis

The prevalence of RA in both the European and American populations ranges from 0.5% to 1%. The prevalence of RA is higher in women than in men, with a two to one ratio. The average age of onset of RA typically between 30 and 60 years. RA can be associated with long-term adverse outcomes, like development of other chronic diseases (cardiovascular and respiratory disease, osteoporosis, malignancies), physical and work disability, psychiatric disorders, reduced quality of life and premature mortality. RA therefore places a significant burden on affected individuals, society and healthcare systems.

1.1.1. Risk factors

The etiology of RA is influenced by genetic factors, as well as lifestyle-related or environmental risk factors. It is well known that presence of cell surface antigens HLA-DR1 and HLA-DR4 are strongly associated with RA. Another major genetic risk factor is variation in the protein tyrosine phosphatase gene (*PTPN22*), which may increase the risk of RA as well as

other autoimmune diseases. Smoking has been found to enhance the citrullination of synovial proteins via peptidyl-arginine deiminase (PAD) enzymes, leading to elevated anti-citrullinated protein antibody (ACPA) levels. Studies have reported a relationship between HLA-DR genes and smoking, suggesting a possible link between the citrullination of proteins in the lungs and T cell-mediated immune activation against them. The gastrointestinal (GI) tract microbiome may also be an important pathogenic factor for RA. Various studies have reported a high prevalence of periodontitis in patients with RA, highlighting the association between these two conditions. Dysbiosis and translocation of gut microbiome may also contribute to autoimmunity. Dysbiosis may lead to loss of barrier function and translocation of gut microbiome via the bloodstream, which may trigger the immune system. Bacterial and viral antigens have been found to have similar amino-acid sequences to autoantigens, which can lead to immune cross-reactivity, called molecular mimicry.

1.1.2. Pathophysiology

As discussed above, genetic and environmental triggers, as well as repeated activation of innate immunity, play a role in the pathogenesis of RA. The PAD-catalysed citrullination of arginine, as described above, as well as carbamylation, acetylation and other protein modifications that generate neoepitopes of autologous proteins, may lead to the production of different autoantibodies, resulting in loss of immunological tolerance. Namely, ACPA and rheumatoid factors (RF) are characteristic antibodies for RA. Altered antibody response to a number of citrullinated proteins induces ACPAs, whereas RF is produced against the Fc portion of immunoglobulins. Furthermore, autoantibodies are able to form immune complexes, that can activate the complement system. Autoantibodies, including ACPAs, RFs and antibodies against carbamylated proteins can be present up to ten years before the onset of clinical arthritis. Neoantigens produced by antigen-presenting cells (APCs) may activate MHC class II-dependent T cells, leading to differentiation of these T cells into effector, memory, and regulatory cells. This process may subsequently trigger the production of ACPAs in B lymphocytes. The immune activation is followed by synovial inflammation. Neovascularization, which is a critical component of the inflammatory process in the synovium, has been demonstrated in RA patients. Due to increased vascular permeability and adhesion molecules, immune complexes accumulate in the joints and induce inflammation,

causing inflammation that presumably contributes to sustained joint inflammation. The synovium is infiltrated by leukocytes, like macrophages, T cells and B lymphocytes, causing an inflammatory cascade. $CD4^+$ T-lymphocytes enhance inflammation by activating synovial macrophages and enhance cartilage destruction and bone erosion through the production of interleukin-17 (IL-17) and tumor necrosis factor α (TNF-α). These inflammatory cytokines further stimulate the expression of Receptor Activator Nuclear Factor κB ligand (RANKL), which in turn leads to the activation of osteoclasts, ultimately resulting in bone resorption. Apart from generating autoantibodies, B cells also release various pro-inflammatory cytokines. These cytokines play a role in the differentiation and activation of T cells. B lymphocytes have been shown to regulate bone homeostasis by expressing RANKL, which increases osteoclast activity, however B cell precursors can produce osteoprotegerin (OPG), which is an inhibitor of osteoclast differentiation.

Macrophages are actively involved in the development of RA and exert their effects through various mechanisms. Synovial macrophages produce cytokines, such as IL-1, IL-6, IL-10, and TNF-α. These cytokines contribute to inflammation, stimulate fibroblast-like synoviocytes (FLS), activate B lymphocytes, and promote the formation of osteoclasts. Synovial macrophage infiltration correlates with radiological progression and ACPA can also enhance TNF-α production in macrophages. FLSs play a pivotal role in the pathogenesis of RA by promoting synovial hyperplasia and producing enzymes, including matrix metalloproteinase 1 (MMP-1) and 13 (MMP-13), which are involved in tissue degradation and joint damage. They also secrete inflammatory cytokines and upregulate the expression of RANKL, which collectively contribute to the degradation of bone and cartilage in RA. Moreover, FLS cells are able to migrate from one joint to the other, which could explain the symmetrical distribution of joint inflammation observed in RA. The synovium also contains neutrophils that generate reactive oxygen species (ROS), proteases and extracellular traps (NETs) consisting of released DNA complexes that contain citrullinated proteins.

1.2. Signs, symptoms and diagnostics

Clinically, RA is characterized by symmetric synovitis mainly affecting the wrists, metacarpophalangeal (MCP), and proximal interphalangeal (PIP) joints. However, other joints,

such as metatarsophalangeal (MTP) joints, knees, elbows, shoulders and temporomandibular joints may also be affected. Patients with RA have fatigue, muscle pain and morning stiffness of joints, which often lasts for more than 30 minutes. Extra-articular manifestations and chronic comorbidities may develop if inadequately treated. Generalized bone loss has also been observed in patients with RA, including osteoporosis, periarticular osteopenia, local bone erosions and periodontal bone loss. RA may lead to the emergence of skin manifestations and in some cases to the development of vasculitis. The most common respiratory manifestations linked to RA are interstitial lung disease (ILD) and pleural disease. Pericarditis and myocarditis are rare in RA, but due to accelerated atherosclerosis, the risk of CVD and the incidence of atrial fibrillation and heart failure are increased in RA patients.

Early detection RA and initiation of treatment is critical to the management of the disease, but establishing a diagnosis of RA can be challenging. ACPA can be detected several years before disease onset and is positive in approximately 67% of RA patients, while RF is less specific but can be detected in up to 80% of RA patients. Both antibodies and higher acute-phase reactants are associated with worst radiographic progression and high disease activity. Plain radiographs are able to detect marginal bone erosions, however in the first year of the disease, erosions of PIP and MCP joints can be found in only 15-30% of patients. MRI can detect bone marrow edema and early erosions. Ultrasonography is another tool to detect synovitis and erosions, and can also be used to assess bursae and ligaments.

1.3. Management

1.3.1. The treat-to-target approach

Early identification and initiation of treatment is crucial in managing patients with RA. Unfortunately, there is no cure for RA, however with modern pharmacological and non-pharmacological interventions we are able to reduce inflammation, pain, decrease joint destruction and prevent long-term disability. The treatment strategy, in addition to the management approach, is extremely important as well. The treat-to-target (T2T) approach aims to achieve remission or low disease activity as treatment goal. The T2T strategy involves achieving a 50% improvement in DAS28 within three months and aiming to attain remission

or low disease activity within six months. T2T also includes the followings: setting a therapeutic target, assessing the target, adapting medication if needed and shared-decision making with the patient. Current EULAR and ACR management guidelines recommend T2T approach, which involves symptomatic treatment and disease modification therapies as soon as possible.

1.3.2. Treatment options

Glucocorticoids (GCs) and non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used in the management of RA. EULAR and ACR management guidelines recommend short-term low-dose GC therapy and GCs tapering as soon as possible. GCs should be considered only during flares or exacerbations, as well as upon starting a new DMARD therapy.

Disease-modifying antirheumatic drugs (DMARDs) represent the most efficacious therapies for the management of RA. They can be divided into three groups: conventional synthetic DMARDs (csDMARDs), biologic DMARDs (bDMARDs) and targeted synthetic DMARDs (tsDMARDs), such as JAK inhibitors. According to the 2021 ACR guideline, methotrexate is considered the primary choice of initial csDMARD therapy for treatment-naive RA patients, whether used in monotherapy or in combination with other csDMARDs. When low disease activity or remission is not reached with first-line treatments, additional bDMARDs or tsDMARDs should be considered as second-line.

1.3.3. JAK-STAT signaling pathway

The JAK family contains four tyrosine kinases: JAK1, JAK2, JAK3 and non-receptor tyrosine kinase protein 2 (TYK2). These molecules are transducers of cytokine signaling, utilizing various cellular processes, including hematopoiesis, lymphocyte proliferation, differentiation, migration, apoptosis and innate antiviral responses. The JAK/STAT signaling pathway can be activated by multiple cytokines, and the specific combination of JAKs and STATs that are activated depends on the ligand and receptor involved.

The blockade of the JAK/STAT pathway presents a novel treatment approach in the therapy of RA, whereby JAK inhibitors (Jakinib) are able to stop this pathway and block the cytokines that

use it. Tofacitinib is an oral small-molecule JAK inhibitor that effectively targets JAK1 and JAK3, with lesser inhibitory effect on JAK2. JAK inhibitors have showed both safety and efficacy in patients with RA, with four Jakinibs, including tofacitinib, being approved for managing RA. Tofacitinib was the first Jakinib approved by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for patients with moderate to severe RA, and can be given orally twice daily as monotherapy or in combination with MTX. Tofacitinib can inhibit the signaling pathways of numerous cytokines, including IL-12, members of the IL-6 family, IL-23, interferons, γ-chain cytokines, and others. In clinical trials, tofacitinib prevented the progression of radiographic joint damage. Baricitinib is an orally administered tsDMARD, which inhibits both JAK1 and JAK2. It showed improvement in clinical outcomes and decreased radiological progression. Both in monotherapy and in combination with csDMARD, upadacitinib, a selective JAK1 inhibitor, has demonstrated significant efficacy in patients who have shown an inadequate response to csDMARD or bDMARD.

1.4. Accelerated atherosclerosis

Traditional risk factors, such as smoking, physical inactivity, hypercholesterolemia, and hypertension, do not fully explain the increased CV morbidity and mortality rates observed in patients with RA. Studies have provided evidence for an increased prevalence of subclinical atherosclerosis in RA patients. Early signs of atherosclerosis, such as endothelial dysfunction and thickening of carotid artery intima-media (ccIMT), have been observed in patients with early RA. Importantly, effective treatment has shown promise in reversing these early signs of atherosclerosis.

Increased systemic inflammation may be the key mechanism connecting RA to increased CV risks and accelerated atherosclerosis. Indeed, both RA and atherosclerosis share common pathophysiological features and underlying mechanisms. In RA and atherosclerosis, endothelial activation, collagen degradation, accumulation of inflammatory cells, and neovascularization are observed. The inflammatory cells involved, particularly monocytes/macrophages and T lymphocytes, play a crucial role in the pathogenesis. These cells produce pro-inflammatory cytokines like IL-6 and TNF-α, which not only enhance the infiltration of inflammatory cells into the intimal layer of blood vessels, but also stimulate the

expression of MMPs, resulting in joint destruction and vascular extracellular matrix degradation. Therefore, the shared inflammatory processes contribute to the pathogenesis of both RA and atherosclerosis, linking these two conditions at cellular and molecular level. IL-6 and TNF-α suppress the production of cyclooxygenase-1 and nitric oxide, resulting in endothelial dysfunction.

Autoantibodies have been linked to CVD as well. ACPAs have been found in atherosclerotic plaques and are associated with increased coronary calcification and atherosclerosis regardless of traditional CV risk factors. NETs may also contribute to atherosclerosis and RA via sustaining inflammation.

1.5. Bone loss

RA has been associated with both generalized bone loss and localized inflammatory bone resorption. The prevalence of osteoporosis in RA is approximately 30%, which is twice as high as that observed in the general population. RA patients have reduced bone mineral density (BMD), increased cortical porosity and an almost doubled risk for vertebral and hip fractures compared to healthy individuals. Long disease duration, high disease activity and increased levels of bone biomarkers are additional factors that contribute to the risk of osteoporosis and fractures in RA. However, recent studies showed that local and systemic bone loss may occur prior to the clinical onset.

1.5.1. RANK-RANKL system

Normal bone remodeling is maintained by the balance between the activities of osteoblasts and osteoclasts. Osteoblasts, originating from mesenchymal precursor cells, have a crucial role in synthesizing and mineralizing bone tissue. On the other hand, osteoclasts, derived from hematopoietic stem cells, contribute to bone resorption by secreting catalytic enzymes and acids, that degrade the bone matrix. Bone turnover may be monitored by different bone biomarkers, such as osteocalcin (OC), procollagen type I N-propeptide (P1NP), C-terminal telopeptide (CTX), and cathepsin K (CATHK). OC, which is secreted solely by osteoblast, and P1NP, which is cleaved from procollagen type I and synthesized by osteoblasts

and fibroblasts, are well known markers of bone formation. Meanwhile, CTX is released during enzymatic bone matrix degradation and CATHK is secreted by activated osteoaclasts, which makes them potential markers of bone resorption. Osteoblasts regulate osteoclast differentiation and activation through RANK-RANKL system. RANKL regulates the formation and function of osteoclasts through its binding to RANK receptors found on both osteoclast precursors and mature cells, facilitated by macrophage colony-stimulating factor (M-CSF). Synovial fibroblasts, activated B cells and T lymphocytes, as well as osteoblasts, are key contributors to the production of RANKL. While RANKL promotes osteoclast proliferation, differentiation and maturation, osteoprotegerin (OPG) represses this process by inhibiting the interaction between RANKL and its receptor and therefore regulating osteoclastogenesis. OPG is a member of TNF receptor superfamily and acts as a soluble decoy receptor for RANKL. OPG is secreted by osteoblasts and inhibits osteoclast maturation and differentiation by blocking the interaction between RANKL and its receptor. OPG-deficient mice developed severe osteoporosis due to excessive bone resorption, while transgenic overexpression of OPG leads to osteopetrosis in mice because they are unable to form osteoclasts. OPG therefore plays an important role in the inhibition of bone resorption. The disruption of the OPG/RANKL ratio directly impacts bone loss in inflammatory conditions. Inflammatory bone loss and bone resorption are linked to pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, IL-17 and IL-23, as well as autoantibodies and the RANK-RANKL system. IL-6 and TNF-α have been reported to directly affect both bone degradation and formation in RA. TNF-α promotes osteoclastogenesis, upregulates RANKL expression on osteoclasts and lymphocytes, and suppresses bone formation by inducing the production of DKK-1. Moreover, TNF-α may inhibits osteoblastogenesis and enhances osteoclast activity via increasing oxidative stress, leading to accelerated bone loss. In RA, treatment with TNF-α inhibitors has been reported to elevate levels of OC and P1NP, and reduce the levels of CTX and RANKL. Furthermore, TNF-α inhibitors increase the ratios of OPG/RANKL, P1NP/CTX and OC/CTX, promoting a favorable balance in bone turnover. T lymphocytes (Th1, Th2, Th17, and Treg) also have a key role in modulating bone remodeling in RA, thus Treg, Th1 and Th2 cells inhibit osteoclastogenesis.

1.5.2. Wnt signaling pathway

Besides the RANK-RANKL system, the Wingless (Wnt) signaling pathway is implicated in the activation and differentiation of osteoblasts and osteoclasts, as well as other functions during embryogenesis. Wnt signaling pathways regulate osteoblastogenesis, osteoblast maturation, OPG synthesis, hence bone formation by transmitting signals through cell surface receptors and activating transcription factors. Activation of the Wnt pathways stimulates osteoblastogenesis and induces the production of OPG in osteoblasts. OPG acts as a decoy receptor for RANKL, thereby inhibiting RANKL-induced osteoclastogenesis and bone resorption. There are two well-known endogenous inhibitors of the Wnt-β-catenin signaling pathway, sclerostin (SOST) and Dickkopf-related protein 1 (DKK1), which are able to bind to Wnt co-receptor low-density lipoprotein receptor-related protein 5 (LRP5) or 6 (LRP6) leading to decreased bone formation. By increasing RANK/RANKL interactions, DKK1 promotes osteoclast differentiation. SOST and DKK-1 may affect each other directly, as DKK-1 inhibition reduces SOST levels in mice. SOST also enhances bone resorption through an autologous effect on osteocyte RANKL production. Studies have revealed that RA patients have increased RANKL and decreased OPG levels in their synovial fluids when compared to OA patients. Additionally, RA patients exhibit a decreased OPG/RANKL ratio. Reports have demonstrated that RA patients with an elevated risk of erosions have higher DKK-1 levels in their synovial fluid and serum.

1.5.3. Autoantibodies in bone loss

Autoantibodies in RA appears to have a significant impact on the mechanism of bone loss. RA patients with ACPA positivity exhibit more severe osteopenia, bone erosions, and more aggressive disease progression, both clinically and radiologically, compared to ACPA negative patients. The presence of ACPA may enhance osteoclast activation and differentiation prior to the onset of arthritis. ACPA may bind to citrullinated vimentin and induce the production of CXCL8 leading to osteoclast differentiation. The process of osteoclast differentiation can also be induced by immune complexes of ACPA and their targets. Citrullination of proteins by PAD is crucial for the differentiation of osteoclasts from macrophage precursors. It was showed that ACPAs increased osteoclastogenesis on a PAD-

dependent autocrine effect of IL-8, furthermore the inhibition of IL-8 decreased osteoclast differentiation. In vivo transfer of ACPAs to mice resulted in a significant bone loss, which was reversed by reparixin, an IL-8 receptor inhibitor. Other autoantibodies, such as anti-carbamylated proteins, have been linked to lower systemic BMD in early arthritis patients.

1.6. Imaging methods for the assessment of vessels and bone

1.6.1. Ultrasound-based techniques

Ultrasound-based methods have been employed to identify underlying vascular abnormalities in patients with RA, which may not have presented clinically yet. Arterial endothelial dysfunction can be assessed noninvasively by an ultrasound-based method, which measures endothelium-dependent flow-mediated dilatation (FMD) of the arterial diameter caused by increased shear-stress. The thickening of the carotid artery's intima-media layer (IMT) and the existence of plaques in the carotid artery are identified as signs of CVD. The speed of arterial pressure waves can be measured using arterial pulse wave velocity (PWV). Studies have found association between PVW and coronary atherosclerosis. According to studies, RA is associated with enlarged carotid IMT, abnormal arterial FMD, and increased PWV, all of which are indications of the subsequent development of CV events.

1.7.2. ^{18}F-FDG-PET/CT

^{18}F-fluorodeoxyglucose-positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) has the potential to detect tissue inflammation throughout the whole body simultaneously. Hence, this imaging method may be utilized to evaluate both vascular and synovial inflammation within the same patient. Patients with ankylosing spondylitis (AS), psoriatic arthritis (PsA), and RA have shown increased FDG uptake in the arterial wall. PET/CT can also detect and monitor vascular inflammation in large-vessel vasculitis.

1.7.3. DXA and QCT

The estimated prevalence of osteoporosis in patients with RA is around 30%. Dual-energy X-ray absorptiometry (DXA) is considered the standard method for assessing BMD at the femur and lumbar spine in osteoporosis. Peripheral quantitative computed tomography (QCT) allows for the measurement volumetric (3-dimensional) BMD, as well as the assessment of cortical and trabecular bone, in addition to bone microarchitecture. In contrast, DXA is limited to assessment of areal (2-dimensional) BMD. QCT is utilized for measuring BMD in peripheral areas of the body, such as legs or forearms.

2. Aims

The aim of this thesis was to gain an understanding of the effects of JAK inhibition on vascular and bone status in patients with RA. We have performed a 12 months follow-up study to investigate the therapeutic effects of tofacitinib on bone metabolism and bone density, as well as aortic and joint inflammation. Although targeted therapies have shown potential benefits on bone remodeling, vascular and systemic inflammation, no PET-CT studies have included JAK inhibitors yet. Furthermore, no prospective studies have been conducted using PET/CT imaging to simultaneously assess both synovial and vascular inflammations in patients with RA.

Primary aims:

- To assess vascular and joint inflammation by ^{18}F-FDG-PET/CT imaging method
- To examine changes on bone metabolism
- To assess bone status and bone mineral density by DXA and QCT
- To assess vascular physiology by ultrasound
- To examine changes on disease activity

Secondary aims:

- To correlate bone mineral density and laboratory biomarkers
- To correlate vascular and synovial inflammation with each other
- To correlate vascular and synovial inflammation with bone biomarkers
- To correlate vascular and synovial inflammation with parameters of bone status
- To correlate vascular and synovial inflammation with disease activity
- To correlate vascular and synovial inflammation with parameters of vascular physiology

3. Patients and methods

3.1. Patient characteristics

A total of 30 patients, consisting of 27 women and 3 men, were enrolled prior to tsDMARD therapy. They were selected based on having a DAS28-CRP score of ≥3.2, indicating moderately active disease. Patients ages 18-80 years, the mean age was 52.8±10.0 (range: 27-69) years and the mean disease duration was 7.7±5.0 (range: 1-21) years. The eligibility criteria involved a definitive diagnosis of RA in accordance with the 2010 EULAR/ACR classification criteria for RA [196]; patients had to have a DAS28 of at least 3.2 at baseline and clinical indication of targeted therapy. Enrolled patients were either treatment-naïve to tsDMARDs (n=16) or previously received maximum one biologic DMARD therapy (n=14). Tofacitinib therapy was initiated following the discontinuation of bDMARD treatment and an appropriate washout period. Patients were ineligible if they had acute or recent infection, any inflammatory disease apart from RA, chronic renal or liver failure, contraindications to JAK inhibition, current use of anti-osteoporotic drugs (bisphosphonates, calcitonin, teriparatide, denosumab), uncontrolled CVD or hypertension, and malignancy within the past 10 years. None of the patients had known primary osteoporosis prior to the diagnosis of RA and altogether 10 patients in the 5 mg and 6 patients in the 10 mg subgroups had received vitamin D supplement therapy at the time of inclusion. However, the dose of vitamin D supplementation remained unchanged throughout the study. Although many of the patients may have been on corticosteroids previously, all patients had discontinued corticosteroid use for a minimum of three months before the study.

Patients were randomly assigned to receive tofacitinib twice daily (bid) in a 1:1 ratio, either at a dose of 5 mg or 10 mg. In addition to tofacitinib, all patients were also receiving concurrent csDMARD therapy, which included methotrexate (n=17), leflunomide (n=5), or sulfasalazine (n=3). The doses of the concomitant csDMARD therapies had remained stable for at least one year prior to the study, and no modifications in the doses were permitted throughout the duration of the study. Laboratory measurements and clinical evaluations were conducted at baseline, at month 6 and month 12, while FDG-PET/CT examinations were assessed at baseline and after 12 months. Eventually four patients (two on each arm)

completed the 6-month follow-up but did not complete the treatment for the full one-year duration. Two patients discontinued due to treatment inefficacy; one had elevated transaminases; and one patient relocated abroad. The data analysis included only those patients who successfully completed the one-year treatment period. A total of fourteen patients had hypertension; two had diabetes mellitus; and seven were current smokers at the time of enrollment.

3.2. Clinical assessments

Clinical evaluations were conducted at baseline, as well as at 6 and 12 months after initiating tofacitinib therapy. A thorough medical history was obtained using a questionnaire, which included inquiries about current smoking status, chest pain, hypertension, CVD, fragility fractures, and diabetes mellitus within the two years preceding the study. This was followed by thorough physical examination and calculation of disease activity using DAS28-CRP (3 variables). The functional capacity of the patients was assessed using the Health Assessment Questionnaire (HAQ).

3.3. Laboratory measurements

Blood samples were collected from fasting patients at baseline, as well as at 6 and 12 months after initiating the therapy. After being drawn into ethylene-diamine-tetraacetate (EDTA)-treated tubes and promptly processed, the samples were divided into aliquots and stored at temperature of -70°C until they were ready to be utilized.

Serum levels of IgM rheumatoid factor (RF) and high sensitivity CRP (hsCRP) were quantitatively measured by nephelometry (Cobas Mira Plus, Roche Diagnostics, Basel, Switzerland), using RF and CRP reagents (both Dialab, Budapest, Hungary). The detection of ACPA (aCCP) autoantibodies in serum samples was performed using a second-generation Immunoscan-RA CCP2 ELISA test (Euro Diagnostica, Malmö, Sweden). The assay was conducted according to the instructions provided by the manufacturer.

Lipids, lipoproteins and apoproteins, including triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol

(TC), lipoprotein a [Lp(a)], apoprotein A (ApoA) and B (ApoB) were determined using routine laboratory methods.

Serum levels of 25-hydroxy-vitamin D3 (25OHVITD3; DiaSorin); phosphate (P; Roche Diagnostics); calcium (Ca; Roche Diagnostics); parathyroid hormone (PTH; Roche Diagnostics); sclerostin (SOST; Biomedica); C-terminal collagen crosslinks (CTX; Roche Diagnostics), procollagen 1 N-terminal propeptide (P1NP; Roche Diagnostics); cathepsin K (CATHK; Biomedica), and osteocalcin (OC; Roche Diagnostics) were determined by ELISA. Levels of Dickkopf-1 (DKK1); osteoprotegerin (OPG) and soluble RANKL were assessed by flow cytometry using a custom multiplex bead immunoassay kit (LEGENDplex, BioLegend) and analyzed by LEGENDplex software. All measurements were carried out at three time points: at baseline, at 6 months after treatment initiation, and 12 months after treatment initiation.

3.4. Assessment of bone mineral density

In order to determine areal BMD of the hip bones and lumbar spine, DXA assessment was performed by a single technician during the study period, using the LUNAR Prodigy densitometer (GE-Lunar Corp., Madison, WI, USA). At our institute, the coefficient of variation of the technique was 0.8% as determined by measuring the anatomical spine phantom daily, and no machine drift was observed throughout the study. The short-term in vivo precision error for L2-L4 lumbar spine is 0.012 g/cm^2 (LSC = 0.034 g/cm^2 at 95% confidence level) and femur neck is 0.013 g/cm^2 (LSC = 0.035 g/cm2 at 95% confidence level). Single-slice quantitative computed tomography (QCT) of the ultra-distal region of the dominant forearm were performed in order to determine volumetric (3D) BMD, using a Stratec XCT-2000 instrument (Stratec Medizintechnik GmbH, Pforzheim, Germany). QCT is able to differentiate between trabecular and cortical bone. Cortical, trabecular and total BMD values obtained through QCT were reported in mg/cm^3. The imaging acquisition was performed with a voxel size of 0.59 mm, and the analysis was conducted using XCT6.00B software (Stratec Medizintechnik GmbH, Pforzheim, Germany).

3.5. ^{18}F-FDG-PET/CT assessments

All patients underwent ^{18}F-FDG-PET/CT (Philips Gemini TF) examination after fasting for at least 6 hours and serum glucose level check. Whole-body scans were performed using the AnyScan PC system (Mediso Medical Imaging Systems, Budapest, Hungary) from the base of the skull to the level of the knees. The scans were obtained two hours after intravenous administration of the 18F-FDG radiopharmaceutical (4.4 MBq/kg). After a visual assessment of the PET and CT images, quantification of vascular inflammation was conducted. Two-dimensional circular regions of interest (ROIs) were drawn around the external aortic contour and merged into tube-like volumes of interest (VOIs) outlining 5 predefined aortic segments (ascending aorta, aortic arch, descending thoracic aorta, suprarenal, and infrarenal abdominal aorta) using dedicated analysis software (InterView FUSION, Mediso, Budapest, Hungary) to determine maximum (SUVmax) and mean standardized uptake values (SUVmean). The mean target-to-background ratio (TBRmean) and the maximum target-to-background ratio (TBRmax) are widely utilized parameters to globally assess vascular inflammation. Aortic TBR-VASCmax and TBR-VASCmean values were obtained by dividing SUV-VASCmax or SUV-VASCmean values of the aortic segments by the SUV_{mean} value of the superior vena cava (blood pool). The mean metabolic volumetric product (MVP_{mean}) was calculated by multiplying SUV_{mean} by VOI volume (cm^3) for each segment as previously reported [166]. To quantify synovial inflammation, SUV-SYNmax and SUV-SYNmean values were determined in VOIs placed with the help of the CT structural images around 5 predefined articular regions (hand/wrist, elbow, shoulder, hip and knee) on both sides, and liver $SUV_{meanliv}$ values were determined and used as reference values. Therefore, the degree of synovial inflammation was expressed as SUV-SYN_{max}/$SUV_{meanliv}$ ratios of each articular region (SUV-$SYN_{mean/liv}$). Finally mean (±SD) of the five TBR-VASCmax and TBR-VASCmean values in the five predefined aortic segments, as well as the mean (±SD) of the five SUV-SYNmean and SUV-SYNmean/liv values in the five articular regions, were calculated.

3.6. Assessment of vascular physiology

3.6.1. Flow-mediated vasodilation, intima–media thickness, pulse wave velocity

Brachial artery flow-mediated dilatation (FMD) and carotid artery intima media thickness (ccIMT) was assessed by using duplex ultrasound (HP Sonos 5500) according to the user manual. The TensioClinic arteriograph system (Tensiomed Ltd, Budapest, Hungary) was used to measure the pulse wave velocity (PWV). The same investigator conducted FMD, IMT and PWV assessments at baseline and after 12 months of tofacitinib therapy.

3.7. Statistical analysis

We utilized various methods to perform statistical analysis through SPSS version 22.0 (IBM, Armonk, NY, USA) software. Continuous variables were expressed as mean ± SD, while categorical variables were expressed as percentages. Both parametric and non-parametric methods were employed. We evaluated the distribution of continuous variables using the Kolmogorov-Smirnov test. Wilcoxon tests and paired two-tailed t-tests were used to measure the statistical significance of continuous variables. Nominal variables, on the other hand, were compared through $\chi 2$ or Fisher's exact test, as appropriate. To assess correlations, Pearson's analysis was utilized. Regression analyses were conducted to investigate the independent associations between various parameters, such as inflammatory, clinical, bone and vascular, as independent variables and PET/CT parameters, as dependent variables. Additionally, univariable and multivariable regression analysis, employing the stepwise method, were utilized to examine the relationships between laboratory and clinical parameters (independent variables) and BMD as assessed by QCT and DXA (dependent variables). The β standardized linear coefficients showing linear correlations between two parameters were determined, and the B (+95% CI) regression coefficient indicated independent associations between dependent and independent variables during changes. Multivariate analysis of variance (MANOVA) was conducted to evaluate the impact of independent variables on two dependent variables simultaneously, while repeated measures analysis of variance (RM-ANOVA) was used to determine the further effects of various parameters including therapy

on 12-month changes of BMD and PET/CT parameters. In these analyses, partial η^2 is given as indicator of effect size, with values of 0.01 suggesting small, 0.06 medium and 0.14 large effect.

4. Results

4.1. Effects of tofacitinib treatment on bone metabolism in RA

4.1.1. Patient characteristics and clinical response to tofacitinib therapy

Four out of the thirty patients did not complete the study; two participants from each group withdrew after six months of therapy. The reasons for discontinuation varied, and included treatment inefficacy, elevated transaminase levels, and discontinuation of study visits. The analysis included a total of twenty-six patients, with thirteen from each group, who completed the study.

JAK inhibition effectively reduced disease activity and systemic inflammation, as showed by the decrease in CRP levels in both groups. In the full cohort (n=26), DAS28 showed a significant decrease from 5.05±0.77 at baseline to 3.31±0.91 ($p<0.001$) after 6 months and to 3.32±1.12 ($p<0.001$) after 12 months of therapy. Additionally, DAS28 score significantly reduced after 6 months (3.23±0.54; $p<0.001$) and after 12 months (3.05±0.77; $p<0.001$) compared to baseline (4.80±0.69), in the 5 mg subset. In the 10 mg group, DAS28 score at baseline, after 6 and 12 months, was 5.29±0.79, 3.39±1.19 ($p<0.001$) and 3.58±1.36 ($p<0.001$), respectively. In the full cohort of patients, tofacitinib therapy led to a reduction in CRP levels from 14.8±14.9 mg/l at baseline to 5.3±5.3 mg/l ($p<0.001$), following a 6-months period of therapy, and to 7.4±7.7 mg/l ($p=0.001$) after 12 months of treatment. The CRP levels significantly changed from 13.3±9.7 mg/l at baseline to 5.3±3.7 mg/l ($p=0.002$) at month 6, and to 7.1±4.0 mg/l ($p=0.022$) at month 12, in the 5 mg tofacitinib group. Furthermore, levels of CRP were 16.3±18.9 mg/l at baseline, 5.2±6.7 mg/l ($p=0.016$) after 6 months, and 7.7±10.3 mg/l ($p=0.014$) after 12 months, in the 10 mg group. The HAQ disability index was used to assess the functional capacity of the patients. In the full cohort significant improvement was seen in HAQ from baseline to month 6, 1.38±0.58 to 1.02±0.67 ($p=0.001$), and at month 12 to 1.02±0.71 ($p=0.001$). In the 10 mg group, HAQ value improved after 6 months (1.10 ± 0.74; $p = 0.010$) and after 12 months (1.15 ± 0.73; $p = 0.005$) compared to baseline (1.59±0.50). However, there was a non-significant trend towards improvement in HAQ score in the subset receiving 5 mg bid tofacitinib.

4.1.2. Effects of tofacitinib therapy on bone loss and bone biomarkers

After 12 months of therapy, tofacitinib demonstrated that it may effectively able to prevent additional bone loss in RA patients. No significant changes were observed in the areal BMD of femoral neck (DXAFNBMD) and vertebrae of L2-4 (DXAL24BMD) over the one-year period in the full cohort and the subgroups receiving 5 mg or 10 mg tofacitinib (p =NS), determined by DXA. Using QTC, there were no significant changes in the cortical (QCTCORTBMD), trabecular (QCTTRABBMD), and total (QCTTOTBMD) volumetric BMD between baseline and the 12-month time point in the full cohort, as well as in the 5 mg and 10 mg subsets (p=NS). One-year tofacitinib treatment resulted changes in areal BMD between −1.5% and 0.1% in the 5 mg subgroup, −0.2% and 1.4% in the 10 mg tofacitinib subset and −0.9% and 0.7% in the full cohort. Changes in volumetric BMD were between −8.1% and 8.2% in the 5 mg subset, −1.5% and 4.9% in the 10 mg subgroup and −4.9% and 6.6% in the full cohort.

According to bone biomarkers, we have measured 12 bone turnover markers. We have observed significant increase in the levels of OC from baseline to 6 months (p=0.013), but only non-significant enhance was seen at month 12. CTX levels significantly decreased from baseline to 6 months (p=0.009) and 12 months (p=0.003). Furthermore, levels of OPG also increased after 6 months (p=0.006) and 12 months of tofacitinib treatment (p=0.004), as well as levels of 25OHVITD3 from baseline to 6 months (p=0.017) and 12 months (p=0.009). With respect to the 5 mg subgroup, OC levels significantly increased after 6 months of therapy (p=0.027), as well as levels of OPG after 6 months (p=0.005) and 12 months (p=0.002). Additional, vitamin D3 levels significantly increased from baseline to 6 months (p=0.001) and 12 months (p=0.004). Moreover, in the 10 mg bid subset, levels of CTX decreased from baseline to 6 months (p=0.005) and 12 months (p=0.007) and levels of OPG increased after 6 months (p=0.047) and 12 months (p=0.029) of therapy. However, JAK inhibition did not change the levels of RANKL, SOST, DKK1, P1NP and PTH significantly, we have found favourable changes in the P1NP/CTX and OC/CTX ratios, but not in the OPG/RANKL ratio. We have found a significant increase in the P1NP/CTX ratio in total patients group from baseline to 6 months (p=0.002) and 12 months (p=0.001). OC/CTX ratios also increased from baseline to 6 months (p<0.001) and to 12 months (p<0.001). In the 5 mg subgroup P1NP/CTX ratios significantly increased after 6 months (p=0.023) and after 12 months (p=0.013) of tofacitinib

therapy, however we did not find significant changes in the 10 mg subset. Additional, OC/CTX ratios in the 5 mg subset, significantly elevated from baseline to 6 months (p=0.010) and to 12 months (p=0.013). Moreover, in the 10 mg group, OC/CTX ratios also increased after 6 months (p=0.002) and after 12 months (p=0.005) of therapy. When we compared the subgroups, we did not find any significant difference between them.

We have found correlations between laboratory biomarkers and bone mineral density parameters at baseline and after one-year of tofacitinib therapy. In the Pearson's correlation analysis negative correlation was observed between DXAL24BMD-0 and CTX-0, CTX-12, P1NP-12 and OC-12 ($p<0.005$). Moreover, DXAL24BMD-12 negatively correlated with RANKL-0, CTX-0, as well with P1NP-12 and CTX-12 ($p<0.05$). DXAFNBMD-0 showed inverse associations with OC-0, CTX-0, CTX-12, P1NP-12 and OC-12 ($p<0.05$). Similarly, DXAFNBMD-12 negatively correlated with CTX-0, OC-0, CTX-12, P1NP-12 and OC-12 ($p<0.05$). With respect to QCTTOTBMD-0 and QCTTRABBMD-0 both showed negative associations with PTH-12, however QCTCORTBMD-12 inversely correlated with RANKL-0 ($p<0.05$) (Table 2).

The univariable regression analysis suggested that RANKL-0, CTX-0, CTX-12, and P1NP-12 may inversely determine DXAL24BMD-12 ($p<0.05$), while CTX-0 may be a negative determinant of DXAL24BMD-0. OC-0, CTX-0 and the age of patients were negative determinants of DXAFNBMD-0 and DXAFNBMD-12. In addition, P1NP-12 and OC-12 inversely correlated with DXAFNBMD-12 ($p<0.05$). QCTTRABBMD-0 by was negatively determined by DAS28-, QCTTOTBMD-12 by CRP-12, while QCTCORTBMD-12 by RANKL-0 and CRP-12 ($p<0.05$). The multivariable analysis confirmed negative associations between CTX-0 and DXAL24BMD-12, age and OC-0 with DXAFNBMD-0, age, OC-0 and CTX-0 with DXAFNBMD- 12, as well as RANKL-0 and CRP-12 with QCTCORTBMD- 12 ($p < 0.005$).

RM-ANOVA analysis was conducted to examine the independent factors that contributed to the changes in volumetric and areal BMD data over a one-year period, with these BMD measurements serving as the dependent variables in the analysis. Significant effects on changes in DXAL24BMD over a one-year period were observed with tofacitinib therapy when combined with lower levels of CCP-0 or DKK1-0. Furthermore, lower CRP-0 or lower age combined with tofacitinib treatment were found to be significant determinants of 12-months changes in QCTCORTBMD ($p<0.05$).

4.2. Assessment of vascular and joint inflammation by PET/CT in associations with vascular and bone status

4.2.1. The effects of tofacitinib therapy on vascular and synovial inflammation

PWV and FMD showed no significant changes between baseline and after 12 months. In the subset of 5 mg bid group, we have observed a significant increase in carotid IMT after 12 months compared to baseline. However, we did not find significant changes in IMT from baseline to 12 months in the 10 mg subgroup.

Vascular and synovial inflammation was assessed by ^{18}F-FDG-PET/CT. One-year tofacitinib therapy resulted in a significant and simultaneous decrease in synovial and vascular inflammation as visualized by PET/CT. The mean articular SUV-SYN significantly reduced after 12 months (2.55±0.50) of tofacitinib treatment compared to baseline (3.18±1.13; p=0.010). TBR-SYN mean showed a significant decrease from baseline (1.53±0.54) to 12 months (1.12±0.22; p=0.001). Aortic TBR-VASC max reduced from baseline (2.17±0.52) to 12 months (1.80±0.30; p<0.001). A non-significant tendency of reduction of TBR-VASC mean was observed over one year of tofacitinib treatment from baseline (1.29±0.29) to 12 months (1.20±0.20; p=0.170).

4.2.2. Statistical analysis of the effects of tofacitinib on vascular and synovial inflammation

No significant correlations were found between aortic TBR values and articular SUV/TBR. However, there were positive and significant correlations between articular TBR-SYN mean and SUV-SYN mean values with anti-CCP, RF, CRP, IMT, PWV, CTX, RANKL, Lp(a) and L2-4 BMD values determined by DXA (p<0.05) from baseline to 12 months of tofacitinib treatment. Aortic TBR-VASC max and TBR-VASC mean values exhibited variable and positive correlations with PWV, DAS28, P1NP, OC, ESR, and negative correlations with HAQ and L2-4 BMD (p<0.05) after 12 months of therapy compared to baseline.

Synovial inflammation, as assessed by PET/CT, showed positive associations with CTX, Lp(a), CRP, PWV, IMT and inversely associated with DXA L2-4 BMD ($p<0.05$) after 12 months, in the univariable analysis. Aortic inflammation was positively associated with OC, P1NP, DAS28, PWV and negatively with HAQ values. The multivariable analysis confirmed the associations of Lp(a) and synovial inflammation after 12 months of therapy, and vascular inflammation and HAQ, P1NP and DAS28 at different time points ($p<0.05$). The multivariable analysis results confirmed previous findings showing an association between Lp(a) and synovial inflammation after 12 months. Additionally, vascular inflammation was found to be associated with HAQ, DAS28, and P1NP at various time points ($p<0.05$). We aimed to investigate the relationships between synovial inflammation measured by PET/CT and ultrasound-detected vascular pathophysiology, as covariates, with systemic inflammation and disease activity, as independent variables.

In the MANOVA analysis DAS28, CRP, and ESR were found to significantly and independently determine both synovial inflammation and PWV or FMD after 12 months of tofacitinib therapy ($p<0.05$). RM-ANOVA analysis was utilized to assess the combined effects of tofacitinib therapy and additional factors on changes in PET/CT parameters over a one-year period. Significant 12-month changes in articular TBR-SYN mean and SUV-SYN mean were determined by the combination of treatment and higher baseline RANKL levels ($p<0.05$). Additionally, therapy in conjunction with elevated level of ESR or lower values of DXA L2-4 BMD were linked to more significant changes in TBR-VASC mean and TBR-VASC max over one-year period ($p<0.05$).

5. Discussion

Effects of tofacitinib on bone metabolism in RA

Bone loss and cardiovascular diseases are major comorbidities in RA. Patients with RA are more prone to generalized osteoporosis and localized bone resorption. Various studies have shown that biological therapies may decrease the incidence of osteoporosis and periarticular erosions, which have both been linked to RA. Studies have demonstrated that in RA, TNF-α inhibitors can raise serum levels of OC and P1NP, while reducing levels of CTX-I and RANKL, resulting in a favorable balance of bone remodeling. Anti-TNF treatment was found to enhance the P1NP/CTX, OPG/RANKL, OC/CTX ratios, and reduce levels of DKK-1, resulting in increased bone formation. Improvement in bone biomarkers by biologics has been associated with decreased inflammatory markers, including CRP, and improvement of disease activity in RA. TNF-α inhibitor therapy arrested generalized bone loss and improved or preserved BMD was found. It was showed that RA was linked to low rate of hand BMD, and following TNF inhibitor treatment hand BMD remained stable over time. Clinical trials have demonstrated that tofacitinib effectively inhibits localized bone loss and reduces radiographic progression. The potential of tofacitinib to inhibit joint damage was observed even when persistent inflammation was present. JAK inhibition decreased RANKL expression and bone resorption in murine models, inhibited osteoclast differentiation, promoted osteoblast activity and stabilized Wnt-dependent bone formation. Baricitinib also reported to inhibit RANKL-mediated osteoclast activity.

Limited research has been conducted on the effects of tofacitinib on bone metabolism in arthritis. However, our study has shown that tofacitinib prevented the advancement of osteoporosis, as neither the areal nor the volumetric BMD changed over time. Additionally, there were improvements in clinical outcomes and a reduction in systemic inflammation with both doses. The measurement of areal and volumetric BMD was conducted through DEXA and QCT, which can evaluate trabecular and cortical bone loss and determine volumetric BMD in RA. Unfortunately, we were unable to compare our BMD results with any other findings regarding JAK inhibition, as there have been no previous prospective investigations on the impact of tofacitinib on BMD alterations. The degree of areal bone loss at various sites varied from 0.2% to 1.5% in our study, after treatment with tofacitinib, in addition some sites even

showed an increase in BMD after one year of therapy. However, annual bone loss determined by BMD in patients with RA was estimated between 2.5% and 3.9%. In a recent study, long-term bDMARDs/tsDMARDs treatment of RA patients was found to have a protective effect on bone loss, thus BMD remained stable, while individuals on conventional therapy suffered significant bone loss. Unfortunately, this trial did not differentiate between the outcomes of patients treated with tofacitinib and those treated with biologics.

We have found that tofacitinib treatment enhanced bone formation markers, including OPG, OC and 25OHVITD3 levels, while decreased markers of bone resorption, such as CTX levels, leading to a positive balance of bone turnover. We have observed that the 10 mg dosage twice a day led to an elevation of levels of OPG and reduction in CTX levels, whereas these changes were not found in the 5 mg bid subset. Overall, we have found favourable changes in the P1NP/CTX and OC/CTX ratios, which suggest an improvement in bone remodeling balance. Tofacitinib has been reported to dampen the synthesis of RANKL and increase the OPG/RANKL ratio in other trials, as well. Additionally, it has been shown to stabilize the anabolic Wnt proteins β-catenin and OC. We have also found elevated 25OHVITD3 levels in response to tofacitinib treatment, which may be related to the ordinary improvement in functional capacity and physical activity of patients.

We found significant associations in correlation analyses between volumetric and areal BMD at baseline and after 12 months of tofacitinib therapy, and various bone turnover markers, including CTX, P1NP, RANKL, OC and PTH. DXA-measured areal BMD was negatively associated with CTX, P1NP, RANKL and OC, whereas QCT-measured volumetric BMD showed a negative association with RANKL and PT, but no significant correlations were observed with other biomarkers. The relationships between bone biomarkers including P1NP, CTX, OC, RANKL, and volumetric and areal BMD were supported by both univariable and multivariable regression models, indicating these markers may play a significant role in defining BMD measurements. Moreover, some baseline bone markers showed correlations with QCT and DXA BMD measurements after 12 months, suggesting their potential as predictors of BMD changes over the course of 12 months. In addition to bone biomarkers, CRP, DAS28, and age were found to be associated with BMD in both univariable and multivariable analyses. Age was found to be significantly associated with femoral neck BMD, but not lumbar spine BMD. Furthermore, age at baseline was identified as a predictor of femoral neck BMD after 12 months. CRP and DAS28 were found to be inversely linked with volumetric BMD, suggesting

that tofacitinib treatment may be a primary factor in improving RA bone status. The RM-ANOVA analysis was utilized to assess the combined effects of tofacitinib therapy and other biomarkers on changes in BMD. The results of our study revealed that the effects of tofacitinib treatment on BMD changes over a one-year period were influenced by various factors. The combination of tofacitinib treatment with lower levels of DKK1 or anti-CCP antibody predicted changes in DXA L2-4 vertebral BMD. On the other hand, the combination of therapy with lower age or levels of CRP predicted changes in QCT cortical BMD. These findings suggest that autoimmunity, age, as well as bone and inflammatory markers, may all play a role in modulating the effects of tofacitinib on BMD changes. We have found no significant differences between the subsets of patients receiving 5 mg or 10 mg of tofacitinib in relation to bone biomarker alterations or BMD. Due to potential safety concerns associated with the 10 mg bid dose of tofacitinib, it is not approved for the treatment of rheumatoid arthritis in the European Union. Therefore, the 5 mg bid dose of tofacitinib may be recommended as a suitable option for maintaining bone status in RA patients.

Effects of tofacitinib on the vasculature and joints in RA

RA is associated with an elevated risk of ischemic stroke, subclinical atherosclerosis, myocardial infarction, coronary calcification, arrhythmias and metabolic changes. For many years, the risk of CVD in autoimmune conditions has been underestimated, although RA patients have almost two times greater risk of developing CVD compared to diabetes mellitus. Studies have shown that higher TNF-α and IL-6 levels are linked to an elevated risk of heart failure, and targeted therapies might have positive effects on cardiovascular outcomes and metabolism. TNF-α inhibitors have been showed to decrease CV risk in patients with RA. They have showed improvement in dyslipidemia, insulin resistance, platelet activation, level of NT-proBNP, moreover, infliximab had an atheroprotective effect in monocytes. In addition, studies have suggested that bDMARDs may prevent the development of periarticular erosions and osteoporosis, and affect bone turnover in RA. Inhibition of TNF-α has been shown to lead to a decrease in bone resorption and an increase in bone formation.

Due to limited evidence on the effects of JAK inhibition on bone loss and CVD in RA, we conducted a one-year prospective study. The aim was to evaluate vascular and synovial

inflammation using ^{18}F-FDG-PET/CT imaging, and to assess bone status and biomarkers in RA patients undergoing either 5mg or 10mg bid tofacitinib therapy. Our study was the first to simultaneously measure vascular and synovial inflammation in patients with RA receiving JAK inhibitor treatment. Our findings indicated that tofacitinib therapy effectively reduced inflammation (ESR, CRP) and disease activity. Additionally, it was found to improve quality of life based on the assessment of HAQ. Previously, FDG-PET/CT has been shown to be a valuable method for assessing disease activity in patients with RA receiving anti-inflammatory treatment. Beckers et al. found significant associations between FDG uptake, disease activity and levels of CRP in RA. It may also provide an early assessment of the overall involvement of RA in the body. TBR assessment was effective in determining vessel wall inflammation and plaque composition in atherosclerosis. An increased inflammation of arterial wall was found in RA, moreover FDG uptake of arterial wall was associated with inflammatory markers, such as ESR, CRP and disease activity. Our study showed that JAK inhibition led to a significant reduction in mean synovial inflammation (SUV-SYN mean and TBR-SYN mean) and maximal aortic inflammation (TBR-VASC max) in five specific articular and aortic locations. However, no correlations were found between aortic TBR and articular SUV values when vascular and synovial inflammation were simultaneously evaluated using PET/CT. In our study, we observed significant associations between PET/CT parameters related to the joint or the aorta and inflammatory markers, including ESR, CRP and DAS28. However, our study revealed correlations between vascular pathology assessed by ultrasound and PET/CT parameters. Specifically, PET/CT-measured synovial inflammation showed positive correlations with IMT and PWV. Furthermore, ESR and disease activity demonstrated variable correlations with aortic inflammation.

The MANOVA analysis revealed that acute phase reactants and disease activity determined both FMD or PWV and synovial inflammation. Additionally, PWV was found to be correlated with aortic inflammation. This suggests that systemic inflammation could contribute to synovial and vascular inflammation, and vascular pathophysiology. In fact, in RA, disease activity, CRP, and ESR are all major factors that contribute to vascular pathology.

Vascular and synovial inflammation detected by PET/CT were associated with BMD and bone biomarkers, as well as disease activity, vascular pathophysiology and systemic inflammation. In addition, baseline synovial SUVmax greater than 1.65 was identified as a predictive factor for the progression of joint destruction. In our study, we observed that

PET/CT-detected synovial and vascular inflammation is correlated not only with localized bone resorption, but also with generalized osteoporosis. The study revealed a correlation between synovial inflammation and RANKL and CTX, which are markers of bone resorption. Additionally, correlation was found between aortic inflammation and bone formation markers, such as OC and P1NP. Furthermore, we found that JAK inhibition, along with higher baseline levels of RANKL, led to changes in TBR-SYN mean and SUV-SYN mean in the RM-ANOVA analysis. Both aortic TBR and synovial SUV/TBR values were negatively correlated to lumbar spine BMD. As a result, systemic inflammation, as well as synovial and vascular inflammation, may contribute to bone loss in RA. There may be an association between atherosclerosis and bone loss, which is exacerbated by arthritis.

JAK inhibition has been linked to increased lipid levels, including mean HDL-C and LDL-C levels. Additionally, those who responded to treatment had higher LDL-C and HDL-C levels than non-responders, and these changes were associated with lower levels of CRP. However, these lipid changes did not have an impact on the atherogenic index or result in any cardiovascular consequences. In this study, the relationship between lipid levels (HDL-C, LDL-C, total cholesterol, and triglycerides) and PET/CT parameters was examined, but no significant association was found between lipids and synovial or aortic inflammation. However, a strong association was observed between Lp(a) and FDG uptake in the synovium, while no association was found in the aortic wall. Previously, Lp(a) has been associated with both rheumatoid arthritis and cardiovascular disease. We have previously discovered a link between Lp(a) and CRP, and biologics were also reported to decrease the synthesis of Lp(a) in RA patients. The mechanism behind the reduction of atherosclerosis caused by tofacitinib remains uncertain, and it is unclear whether the improvement is related to lipid metabolism.

6. Summary

In summary, the treatment with tofacitinib effectively reduced both synovial and vascular inflammation simultaneously as determined by ^{18}F-FDG-PET/CT and attenuated the further development of bone loss in RA. Tofacitinib in both doses significantly decreased disease activity, improved clinical outcomes and decreased systemic inflammation. We have found that age, CTX, and OC were independent predictors of areal BMD, while CRP and RANKL were independent predictors of volumetric BMD. CRP, DKK-1, age and ACPA influenced the effects of tofacitinib therapy on BMD changes. Our findings indicate that CRP, IMT, PWV, CTX, RANKL, and Lp(a) could be considered as individual predictors of synovial inflammation. Additionally, HAQ, ESR, PWV, DAS28, OC and P1NP determined aortic FDG uptake. It appears that disease activity and systemic inflammation may play a role in influencing both vascular pathophysiology and synovial inflammation. ^{18}F-FDG-PET/CT may be suitable method for simultaneous assessment of vascular and synovial inflammation, as well as monitoring the effects of anti-rheumatic and other therapies on tissue inflammation.

To the best of our knowledge, this is the first prospective study conducted over one year to examine the effects of tofacitinib therapy on bone health and vascular pathophysiology in RA, in conjunction with disease activity, bone turnover markers, and inflammation. In addition, this may be the first study to evaluate the impact of 12 months of tofacitinib therapy on both aortic and synovial inflammation, as determined by ^{18}F-FDG-PET/CT. Our study contributes to the understanding of the effects of JAK inhibition in RA, but further research is needed to investigate the potential positive effects of tofacitinib and other JAK inhibitors on vascular and joint inflammation in RA.

7. Acknowledgement

First and foremost, I would like to thank my supervisor, Dr. Szilvia Szamosi and Prof. Dr. Zoltán Szekanecz. Their exceptional expertise, constant support and guidance have been invaluable throughout the entire process. I am very grateful for the immeasurable contributions they made to my development. Also, I am very thankful to Prof. Dr. Gabriella Szűcs, Head of the Rheumatology Clinic, for helping me with my work.

I would like to express my special appreciation and thanks to Dr. Zsolt Hascsi, Katalin Hodosi, Anita Pusztai and Dr. Gábor Tajti for their significant contribution to the research.

Furthermore, I am indebted to my colleagues, whose support and encouragement have been a constant source of motivation. I would especially like to thank the nurses and assistants of the Rheumatology Clinic for their support.

Lastly, I want to express my deepest gratitude to my family. Your encouragement and belief played an integral role in my accomplishments.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/433/2023.PL
Subject: PhD Publication List

Candidate: Attila Béla Hamar
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Hamar, A. B.**, Szekanecz, Z., Karancsiné Pusztai, A., Czókolyová, M., Végh, E., Pethő, Z., Bodnár, N., Gulyás, K., Horváth, Á., Soós, B., Bodoki, L., Bhattoa, H. P., Nagy, G., Tajti, G., Panyi, G., Szekanecz, É., Domján, A., Hódosi, K., Szántó, S., Szűcs, G., Szamosi, S.: Effects of one-year tofacitinib therapy on bone metabolism in rheumatoid arthritis.
Osteoporosis Int. 32 (8), 1621-1629, 2021.
DOI: http://dx.doi.org/10.1007/s00198-021-05871-0
IF: 5.071

2. **Hamar, A. B.**, Hascsi, Z., Karancsiné Pusztai, A., Czókolyová, M., Végh, E., Pethő, Z., Gulyás, K., Soós, B., Kerekes, G., Szekanecz, É., Hódosi, K., Szántó, S., Szűcs, G., Seres, T., Szekanecz, Z., Szamosi, S.: Prospective, simultaneous assessment of joint and vascular inflammation by PET/CT in tofacitinib-treated patients with rheumatoid arthritis: associations with vascular and bone status.
RMD Open. 7 (3), 1-10, 2021.
DOI: http://dx.doi.org/10.1136/rmdopen-2021-001804
IF: 5.806

List of other publications

3. Soós, B., Fagyas, M., Horváth, Á., Végh, E., Karancsiné Pusztai, A., Czókolyová, M., Csongrádi, A., **Hamar, A. B.**, Pethő, Z., Bodnár, N., Kerekes, G., Hódosi, K., Szekanecz, É., Szamosi, S., Szántó, S., Szűcs, G., Papp, Z., Szekanecz, Z.: Angiotensin Converting Enzyme Activity in Anti-TNF-Treated Rheumatoid Arthritis and Ankylosing Spondylitis Patients.
Front. Med. 8, 1-11, 2022.
DOI: http://dx.doi.org/10.3389/fmed.2021.785744
IF: 3.9

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

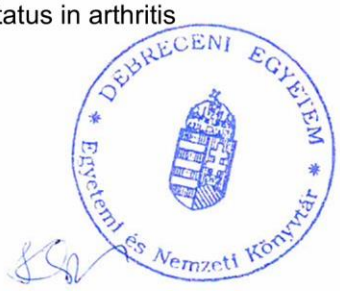
4. Czókolyová, M., **Hamar, A. B.**, Karancsiné Pusztai, A., Tajti, G., Végh, E., Pethő, Z., Bodnár, N., Horváth, Á., Soós, B., Szamosi, S., Szentpéteri, A., Seres, I., Harangi, M., Paragh, G., Kerekes, G., Bodoki, L., Domján, A., Hódosi, K., Seres, T., Panyi, G., Szekanecz, Z., Szűcs, G.: Effects of One-Year Tofacitinib Therapy on Lipids and Adipokines in Association with Vascular Pathophysiology in Rheumatoid Arthritis.
Biomolecules. 12, 1-22, 2022.
DOI: http://dx.doi.org/10.3390/biom12101483
IF: 5.5

5. Soós, B., **Hamar, A. B.**, Pusztai, A., Czókolyová, M., Végh, E., Szamosi, S., Pethő, Z., Gulyás, K., Kerekes, G., Szántó, S., Szűcs, G., Christians, U., Klawitter, J., Seres, T., Szekanecz, Z.: Effects of tofacitinib therapy on arginine and methionine metabolites in association with vascular pathophysiology in rheumatoid arthritis: a metabolomic approach.
Front. Med. 9, 1-16, 2022.
DOI: http://dx.doi.org/10.3389/fmed.2022.1011734
IF: 3.9

6. Nagy, G., Roodenrijs, N. M. T., Welsing, P. M. J., Kedves, M., **Hamar, A. B.**, van der Goes, M. C., Kent, A., Bakkers, M., Pchelnikova, P., Blaas, E., Senolt, L., Szekanecz, Z., Choy, E., Dougados, M., Jacobs, J. W. G., Geenen, R., Bijlsma, J. W., Zink, A., Aletaha, D., Schoneveld, L., van Riel, P., Dumas, S., Prior, Y., Nikiphorou, E., Ferraccioli, G., Schett, G., Hyrich, K. L., Mueller, L. U., Buch, M. H., McInnes, I. B., van der Heijde, D., van Laar, J. M.: EULAR points to consider for the management of difficult-to-treat rheumatoid arthritis.
Ann. Rheum. Dis. 81 (1), 20-33, 2022.
DOI: http://dx.doi.org/10.1136/annrheumdis-2021-220973
IF: 27.4

7. Szekanecz, Z., **Hamar, A. B.**, Soós, B.: A JAK-gátlók biztonságossági kérdései rheumatoid arthritisben.
Immunol. Szle. 13 (1), 4-19, 2021.

8. Karancsiné Pusztai, A., **Hamar, A. B.**, Czókolyová, M., Gulyás, K., Horváth, Á., Végh, E., Pethő, Z., Szamosi, S., Balogh, E., Bodnár, N., Bodoki, L., Szentpétery, Á., Bhattoa, H. P., Kerekes, G., Juhász, B., Szekanecz, É., Hódosi, K., Domján, A., Szántó, S., Raterman, H. G., Lems, W. F., Szekanecz, Z., Szűcs, G.: Associations of vascular and bone status in arthritis patients.
Sci. Rep. 11, 1-10, 2021.
DOI: http://dx.doi.org/10.1038/s41598-021-99071-9
IF: 4.996



UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

9. Czókolyová, M., Karancsiné Pusztai, A., Végh, E., Horváth, Á., Szentpéteri, A., **Hamar, A. B.**, Szamosi, S., Hódosi, K., Domján, A., Szántó, S., Kerekes, G., Seres, I., Harangi, M., Paragh, G., Szekanecz, É., Szekanecz, Z., Szűcs, G.: Changes of Metabolic Biomarker Levels upon One-Year Anti-TNF-α Therapy in Rheumatoid Arthritis and Ankylosing Spondylitis: associations with Vascular Pathophysiology.
Biomolecules. 11 (10), 1-15, 2021.
DOI: http://dx.doi.org/10.3390/biom11101535
IF: 6.064

10. Roodenrijs, N. M. T., Kedves, M., **Hamar, A. B.**, Nagy, G., Laar, J. M. v., Heijde, D. v. d., Welsing, P. M. J.: Diagnostic issues in difficult-to-treat rheumatoid arthritis: a systematic literature review informing the EULAR recommendations for the management of difficult-to-treat rheumatoid arthritis.
RMD Open. 7 (1), 1-16, 2021.
DOI: http://dx.doi.org/10.1136/rmdopen-2020-001511
IF: 5.806

11. Nagy, G., Roodenrijs, N. M. T., Welsing, P. M. J., Kedves, M., **Hamar, A. B.**, Goes, M. C. v. d., Kent, A., Bakkers, M., Blaas, E., Senolt, L., Szekanecz, Z., Choy, E., Dougados, M., Jacobs, J. W. G., Geenen, R., Bijlsma, J. W., Zink, A., Aletaha, D., Schoneveld, L., Riel, P. v., Gutermann, L., Prior, Y., Nikiphorou, E., Ferraccioli, G., Schett, G., Hyrich, K. L., Mueller, L. U., Buch, M. H., McInnes, I. B., Heijde, D. v. d., Laar, J. M. v.: EULAR definition of difficult-to-treat rheumatoid arthritis.
Ann. Rheum. Dis. 80 (1), 31-35, 2021.
DOI: http://dx.doi.org/10.1136/annrheumdis-2020-217344
IF: 27.973

12. Juhász, B., Gulyás, K., Horváth, Á., Végh, E., Karancsiné Pusztai, A., Szentpétery, Á., Pethő, Z., Bodnár, N., **Hamar, A. B.**, Bodoki, L., Bhattoa, H. P., Szekanecz, É., Hódosi, K., Domján, A., Szamosi, S., Horváth, C., Szántó, S., Szűcs, G., Raterman, H. G., Lems, W. F., FitzGerald, O., Szekanecz, Z.: Peripheral quantitative computed tomography in the assessment of bone mineral density in anti-TNF-treated rheumatoid arthritis and ankylosing spondylitis patients.
BMC Musculoskelet. Disord. 22 (1), 1-9, 2021.
DOI: http://dx.doi.org/10.1186/s12891-021-04708-5
IF: 2.562

13. Roodenrijs, N. M. T., **Hamar, A. B.**, Kedves, M., Nagy, G., Laar, J. M. v., Heijde, D. v. d., Welsing, P. M. J.: Pharmacological and non-pharmacological therapeutic strategies in difficult-to-treat rheumatoid arthritis: a systematic literature review informing the EULAR recommendations for the management of difficult-to-treat rheumatoid arthritis.
RMD Open. 7 (1), 1-20, 2021.
DOI: http://dx.doi.org/10.1136/rmdopen-2020-001512
IF: 5.806

14. Karancsiné Pusztai, A., **Hamar, A. B.**, Horváth, Á., Gulyás, K., Végh, E., Bodnár, N., Kerekes, G., Czókolyová, M., Bodoki, L., Hódosi, K., Domján, A., Nagy, G., Szöllősi, I., Lopez, L. R., Matsuura, E., Prohászka, Z., Szántó, S., Szűcs, G., Nagy, Z., Shoenfeld, Y., Szekanecz, Z., Szamosi, S.: Soluble vascular biomarkers in rheumatoid arthritis and ankylosing spondylitis: effects of one-year anti-TNF-[alfa] therapy.
J. Rheumatol. 48 (6), 821-828, 2021.
DOI: http://dx.doi.org/10.3899/jrheum.200916
IF: 5.346

15. Gulyás, K., Horváth, Á., Végh, E., Karancsiné Pusztai, A., Szentpétery, Á., Pethő, Z., Váncsa, A., Bodnár, N., Csomor, P., **Hamar, A. B.**, Bodoki, L., Bhattoa, H. P., Juhász, B., Nagy, Z., Hódosi, K., Karosi, T., FitzGerald, O., Szűcs, G., Szekanecz, Z., Szamosi, S., Szántó, S.: Effects of 1-year anti-TNF-[alfa] therapies on bone mineral density and bone biomarkers in rheumatoid arthritis and ankylosing spondylitis.
Clin. Rheumatol. 39 (1), 167-175, 2020.
DOI: http://dx.doi.org/10.1007/s10067-019-04771-3
IF: 2.98

16. Végh, E., Kerekes, G., Karancsiné Pusztai, A., **Hamar, A. B.**, Szamosi, S., Váncsa, A., Bodoki, L., Pogácsás, L., Balázs, F., Hódosi, K., Domján, A., Szántó, S., Nagy, Z., Szekanecz, Z., Szűcs, G.: Effects of 1-year anti-TNF-α therapy on vascular function in rheumatoid arthritis and ankylosing spondylitis.
Rheumatol. Int. 40 (3), 427-436, 2020.
DOI: http://dx.doi.org/10.1007/s00296-019-04497-0
IF: 2.631

17. Balogh, E., Karancsiné Pusztai, A., **Hamar, A. B.**, Végh, E., Szamosi, S., Kerekes, G., McCormick, J., Biniecka, M., Szántó, S., Szűcs, G., Nagy, Z., Fearon, U., Veale, D. J., Szekanecz, Z.: Autoimmune and angiogenic biomarkers in autoimmune atherosclerosis.
Clin. Immunol. 199, 47-51, 2019.
DOI: http://dx.doi.org/10.1016/j.clim.2018.12.011
IF: 3.368

18. Sarzi-Puttini, P., Marotto, D., Caporali, R., Galeazzi, M., Atzeni, F., **Hamar, A. B.**, Soós, B., Szekanecz, Z.: Biosimilars vs originators: are they the same?
Autoimmun. Rev. 18 (12), 1-5, 2019.
DOI: http://dx.doi.org/10.1016/j.autrev.2019.102404
IF: 7.767

19. Horváth, Á., Végh, E., Karancsiné Pusztai, A., Pethő, Z., **Hamar, A. B.**, Czókolyová, M., Bhattoa, H. P., Nagy, G., Juhász, B., Hódosi, K., Domján, A., Szekanecz, Z., Szűcs, G., Szamosi, S.: Complex assessment of bone mineral density, fracture risk, vitamin D status and bone metabolism in Hungarian systemic sclerosis patients.
Arthritis Res. Ther. 21 (1), 1-10, 2019.
IF: 4.103

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

20. Balogh, E., Végh, E., Kerekes, G., Karancsiné Pusztai, A., **Hamar, A. B.**, Hódosi, K., Szamosi, S., Váncsa, A., Csomor, P., Bodoki, L., Pogácsás, L., Balázs, F., Tar, I., McCormick, J., Biniecka, M., Fearon, U., Lundberg, K., Kharlamova, N., Szántó, S., Szűcs, G., Nagy, Z., Veale, D. J., Szekanecz, Z.: Effects of one-year anti-TNF-[alfa] therapy on biomarkers of angiogenesis and functional vascular parameters in arthritides.
Rheumatol. Orthop. Med. 4, 1-8, 2019.
DOI: http://dx.doi.org/10.15761/ROM.1000169

21. Póliska, S., Besenyei, T., Végh, E., **Hamar, A. B.**, Karancsiné Pusztai, A., Váncsa, A., Bodnár, N., Szamosi, S., Csumita, M., Kerekes, G., Szabó, Z., Nagy, Z., Szűcs, G., Szántó, S., Zahuczky, G., Nagy, L., Szekanecz, Z.: Gene expression analysis of vascular pathophysiology related to anti-TNF treatment in rheumatoid arthritis.
Arthritis Res. Ther. 21 (1), 94, 2019.
IF: 4.103

22. Szekanecz, Z., Tóth, Z., **Hamar, A. B.**, Lánczi, L.: Miért mennek/mentek külföldre a debreceni orvosok?: egy felmérés eredményei.
Orvosi Hetilap. 158 (37), 1458-1468, 2017.
DOI: http://dx.doi.org/10.1556/650.2017.30842
IF: 0.322

23. **Hamar, A. B.**, Karancsiné Pusztai, A., Szántó, S., Szekanecz, Z.: A tirozinkináz-gátlás lehetőségei rheumatoid arthritisben.
Immunol. Szle. 8 (3), 4-21, 2016.

Total IF of journals (all publications): 135,404
Total IF of journals (publications related to the dissertation): 10,877

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

20 September, 2023