Table 2. Ultrasound Characteristics in PsA patients

	CP	Without CP	P
FRS-Lipids, n (%)			0.010
Low and Moderate risk	17(70.8)	39(95.1)	
High risk	7(29.2)	2(4.9)	
FRS-BMI, n (%)			NS
Low and Moderate risk	15(62.5)	32(80)	
High risk	9(37.5)	8(20)	
ACC/AHA 2013, n (%)			<0.001
Low, Borderline and Moderate risk	14(56)	35(97.2)	
High risk	11(44)	1(2.8)	

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Bone diseases, including osteoporosis and osteoimmunology: aetiology, pathology and animal models

FRI0367

ADALIMUMAB IS MORE EFFECTIVE THAN ETANERCEPT AT PREVENTING TNF-ENHANCED OSTEOCLAST DEVELOPMENT THROUGH DOWNREGULATION OF PRO-OSTEOCLASTOGENIC FACTORS ICAM-1 AND IGFBP2 AND UPREGULATION OF ANTI-OSTEOCLASTOGENIC FACTOR FABP4

B. Harvey¹, L. Jin², J. Maull², C. Hu², Y. Tian², Z. Kaymakalan¹. ¹AbbVie Bioresearch Center, Biologics, Worcester, United States of America; ²AbbVie Bioresearch Center, DMPK, Worcester, United States of America

Background: TNF has been shown to contribute to osteoclastogenesis independently and in conjunction with M-CSF and RANKL. We have previously demonstrated that TNF enhances the kinetics of RANKL-induced human osteoclastogenesis and that its effects are mitigated more effectively by the anti-TNF biologic adalimumab (ADA) as compared to etanercept (ETN).

Objectives: To determine the mechanism responsible for the difference in effectiveness between the two biologics, a label-free quantitative proteomics study was conducted on TNF-activated human osteoclasts (OC) upon biologic treatment.

Methods: Human bone marrow-derived OC precursors (OCP, 3 donors) were exposed for 5 days to M-CSF, M-CSF+RANKL (RANKL) alone or in combination with 100 ng/mL TNF +/- 5 ug/mL ADA, ETN or human IgG1 (IGG) as pre-formed complexes. OC differentiation was confirmed by measuring TRAP5b activity. Shotgun proteomics was performed on peptides generated from detergent based cell extracts subjected to methanol/chloroform precipitation and trypsin/Lys-C digestion. Data acquisition was performed with Orbitrap Q Exactive™ HF-X mass spectrometer. MaxQuant was utilized to quantify proteins based on MS1 peak intensities. Data matrix was normalized, imputed and subjected to differential expression analysis by limma. DAVID pathway analysis (DPA) was used to identify pathways impacted by various treatment conditions based on proteins exhibiting significant (p<0.05) 1.2-fold change in expression as compared to RANKL. Protein levels within culture supernatant or cell lysate were verified by ELISA or MSD.

Results: Principle component analysis (PCA) of the proteomic profiling data for the 3 donors indicated that exposure of OCP to TNF induced a distinct profile from that of M-CSF and RANKL. The addition of ADA:TNF complexes restored the profile to that of RANKL, whereas those exposed to ETN:TNF complexes exhibited an intermediate profile matching differences observed in TRAP 5b activity levels. DPA identified 3 pathways most associated with osteoclastogenesis: receptor-mediated endocytosis (e.g. CD163, IGFBP2), oxidation-reduction process (e.g. FABP4), and cell adhesion (e.g. ICAM-1, TGFB1) that were significantly impacted by TNF with ADA:TNF being more effective than ETN:TNF in restoring most pathway-associated proteins to RANKL levels. Based on ELISA, two pro-osteoclastogenic factors IGFBP2 and ICAM-1 were increased 2-fold in OCP culture supernatants in response to TNF with only ADA:TNF complexes reducing these levels of both to RANKL. TNF-induced reduction of intracellular levels of CD163, an M2 macrophage polarization marker, and TGFB1 were not only restored to RANKL levels by ADA:TNF but brought to a level closer to M-CSF alone, unlike ETN:TNF. One anti-osteoclastogenic factor FABP4 was found to be increased intracellularly above that of RANKL only following exposure to ADA:TNF complexes.

Conclusion: Shotgun proteomic profiling of human OCP differentiated *in vitro* with TNF revealed at least 3 novel pathways by which TNF exerts its pro-osteoclastogenic effects. Only with the addition of ADA:TNF, not with ETN:TNF, were most pathway-associated proteins significantly restored to RANKL levels

including pro-osteoclastogenic factors ICAM-1 and IGFBP2. Moreover, ADA:TNF complexes enhanced the intracellular levels of CD163 and the anti-osteoclastogenic factor FABP4, suggesting these complexes are exerting an effect on OCP beyond simple TNF neutralization. Additional *in vitro* and *in vivo* studies need to be performed to verify our findings.

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FRI0368

GLUCOCORTICOID THERAPY MIGHT SUPPRESS WNT SIGNALING BY REDUCING THE RATIO OF SERUM WNT3A TO WNT INHIBITORS, SFRP-1 AND WIF-1, AND IMPAIR BONE FORMATION

M. Kawazoe¹, K. Kaneko¹, T. Nanki¹. ¹Toho University School of Medicine, Division of Rheumatology, Department of Internal Medicine, Tokyo, Japan

Background: Glucocorticoids decrease bone density by multiple mechanisms, including suppression of bone formation via Wnt/β-catenin signaling. Binding of Wnt ligands to a specific receptor and its co-receptors is required for activation of the Wnt pathway, whereas this pathway is inactivated by some negative regulators of Wnt signaling. Sclerostin (Scl) and Dickkopf-1 (Dkk-1) bind to Wnt co-receptors, and secreted Frizzled-related protein 1 (sFRP-1) and Wnt inhibitory factor 1 (Wif-1) bind to Wnt ligand, thereby inactivating the Wnt pathway [1-4]. However, the detailed changes of Wnt signaling in patients with glucocorticoid-induced osteoporosis have not been clarified.

Objectives: We measured serum levels of Scl, Dkk-1 and Wnt3a before and after starting glucocorticoid therapy in our previous study, and the results suggested that suppression of Wnt/β-catenin signaling by increasing serum Scl and Dkk-1 might impaired bone formation at least in the first week of the initiation of glucocorticoid therapy [5]. However, the involvement of Wnt signaling in subsequent suppression of bone formation was unclear. The objective of this study was to investigate the involvement of the Wnt/β-catenin signaling pathway and its clinical significance after the early phase of glucocorticoid therapy in glucocorticoid-induced osteoporosis.

Methods: A total of 53 patients with systemic autoimmune diseases who received initial glucocorticoid therapy with prednisolone (30-60mg daily) were prospectively enrolled. We measured serum levels of sFRP-1, Wif-1 and Wnt3a before starting glucocorticoid therapy and every week for four weeks after its initiation. Patients underwent measurement of bone mineral density (BMD) of the lumbar spine (L2-4) by dual-energy X-ray absorptiometry before starting therapy and after 16.3 ± 1.4 months (the mean ± SEM).

Results: Serum sFRP-1 and Wif-1 level tended to decrease compared to before therapy from the first week. Serum level of Wnt3a also decreased from the first week. Both the ratio of Wnt3a to sFRP-1 and the ratio of Wnt3a to Wif-1 decreased from the first week onward. Moreover, we stratified the subjects into two groups according to the baseline serum sFRP-1 level at median and found that the decrease of BMD after initiation of glucocorticoid therapy in the High sFRP-1 group was larger than that in the Low sFRP-1 group. There was no difference in BMD changes between High Wif-1 and Low Wif-1 group, when stratified into two groups according to the median baseline serum Wif-1 level.

Conclusion: Our previous study indicated that increase of Scl and Dkk-1 could inhibit Wnt signaling pathway in the early phase of glucocorticoid therapy. Current study suggested that the reduction in the ratio of Wnt3a to Wnt inhibitors, sFRP-1 and Wif-1, would suppress Wnt signaling, which might result in impairment of bone formation subsequent. Taken together, bone formation was impaired via suppressing Wnt signaling in patients treated with glucocorticoid. Furthermore, higher serum sFRP-1 level before glucocorticoid administration might be a predictor of future severity of glucocorticoid-induced osteoporosis.

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FRI0369

MIMICKING GLUCOCORTICOID-INDUCED OSTEOPOROSIS USING AN IN VITRO TRABECULAR HUMAN BONE MODEL

A. Lang^{1,2}, K. Diesing¹, A. Damerau^{1,2}, S. Uzun¹, M. Pfeiffenberger^{1,2}, T. Gaber^{1,2}, F. Buttgerit^{1,2}. ¹Charité-Universitätsmedizin Berlin, Department for Rheumatology and Clinical Immunology, Berlin, Germany; ²German Rheumatism Research Center, Berlin, Germany

Background: The bone matrix consists of inorganic and organic components and a variety of specialized cells such as osteoblasts, osteocytes and osteoclasts. The bone-forming osteoblasts are responsible for the production of organic matrix components; they differentiate later into osteocytes which is accompanied by matrix mineralization. Osteoclasts are multinuclear giant cells, which resorb bone. Healthy bone homeostasis is characterized by a balanced, dynamic and continuous remodeling process. Glucocorticoids (GCs) are commonly used to successfully treat patients with inflammatory rheumatic and other autoimmune diseases. However, long-term treatment with GC can potentially lead to several adverse effects such as the inhibition of osteoblast proliferation and the increase of osteoclastic activity resulting in osteoporosis.

Objectives: Hence, the aim of our project is to i) develop an *in vitro* trabecular human bone model, ii) integrate this bone model into a perfusion system to accelerate mineralization and provide biomechanical stimuli and iii) applying prednisolone to induce osteoporosis. Here we present our initial results describing the successful differentiation of osteoblasts and osteoclasts in a 3D environment, and the accomplished integration of the bone model into a perfusion system.

Methods: In a first step, different cultivation conditions were tested to allow optimal osteogenic or osteoclastic differentiation. To this end, a) human bone marrow derived mesenchymal stromal cells (hMSCs) were treated with osteogenic medium, and b) monocytes (isolated from buffy coats) were differentiated into osteoclasts using following protocol: incubation for 3 days with 25 ng/ml M-CSF followed by an 18-day incubation with M-CSF and 50 ng/ml RANKL. Calcification of hMSCs was evaluated via Alizarin Red S staining. Osteoclasts were identified using immunofluorescence staining observing multinucleated (DAPI) giant (β -Actin) cells with TRAP and Cathepsin K activity. Additional gene expression analyses are currently conducted using qRT-PCR and looking for osteoclast-specific genes. In parallel to the monolayer cultures, cells were transferred on β -tricalcium phosphate (β TCP) – a suitable bony-like scaffold. Furthermore, first experiments in a dynamic bioreactor platform (OSPIN GmH) were conducted to evaluate the influence of shear stress on the cells and model systems.

Results: We have been able to populate the β TCP scaffold with monocytes, which were differentiated into osteoclasts (morphological changes) without any effect on cellular viability as measured by Live/Dead staining. The morphological changes of those osteoclasts such as formation of filopodia could be demonstrated by scanning electron microscopy. In addition, the cultivation of β TCP populated with hMSCs in a perfusion system showed the upregulation of osteogenic markers (RUNX2, OSX) on mRNA-level.

Conclusion: These first results of our approach to develop an *in vitro* 3D model for glucocorticoid-induced osteoporosis are promising. Our next step will be the co-cultivation of osteoblasts and osteoclasts under dynamic and optimized cultivation conditions. By combining several cell types, a suitable scaffold and biomechanical stimuli (perfusion), we aim to provide a valid testing platform to study underlying disease mechanisms and for drug development.

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FRI0370

DUAL OXIDASE MATURATION FACTOR 1 POSITIVELY REGULATES RANKL-INDUCED OSTEOCLASTOGENESIS VIA ACTIVATING REACTIVE OXYGEN SPECIES PRODUCTION AND TRAF6-MEDIATED SIGNALING

C. H. Lee¹, C. H. Chung¹, Y. J. Choi², W. H. Yoo³, J. Y. Kim³, M. S. Lee¹.

¹Wonkwang University Hospital, Internal Medicine, Iksan, Korea, Rep. of (South Korea); ²Chonbuk National University Hospital, Internal Medicine, Jeonju, Korea, Rep. of (South Korea); ³Wonkwang University, School of Medicine, Iksan, Korea, Rep. of (South Korea)

Background: Reactive oxygen species (ROS) are one of the significant factors of chemical or physical cell signaling in a wide variety of cell types including skeletal cells. Receptor activator of NF- β B ligand (RANKL) induces generation of intracellular ROS, which act as second messengers in RANKL-mediated osteoclastogenesis. Dual oxidase maturation factor 1 (Duoxa1) was first identified as a *Drosophila* Numb-interacting protein (NIP), and has been associated with the maturation of ROS generating enzymes including dual oxidases (Duox1 and Duox2). In the progression of osteoclast differentiation using mouse bone marrow-derived macrophages (BMMs), we identified that only Duoxa1 level showed an effective change upon RANKL stimulation, but not Duox1, Duox2, and Duoxa2.

Objectives: we hypothesized that Duoxa1 could independently act as a second messenger for RANKL stimulation and regulate ROS production during osteoclast differentiation.

Methods: Using siRNA or retrovirus transduction and knockdown of Duoxa1 via siRNA

Results: Duoxa1 level gradually increased during RANKL-induced osteoclast differentiation. We found that Duoxa1 regulated RANKL-stimulated osteoclast formation and bone resorption positively. Knockdown of Duoxa1 via siRNA decreased the RANKL-induced ROS production. During Duoxa1-related control of osteoclastogenesis, activation of tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6)-mediated early signaling molecules including MAPKs, Akt, β B, Btk, and PLC 2 was affected, which sequentially modified the mRNA or protein expression levels of key transcription factors in osteoclastogenesis, such as c-Fos and NFATc1, as well as mRNA expression of osteoclast-specific markers including OSCAR, ATP6v0d2, and Ctsk.

Conclusion: Overall, our data indicate that Duoxa1 plays a crucial role in osteoclastogenesis via regulating RANKL-induced intracellular ROS production and activating TRAF6-mediated signaling.

Disclosure of Interests: None declared

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FRI0371

BETULINIC ACID INHIBITS RANKL-INDUCED OSTEOCLASTOGENESIS VIA ATTENUATING AKT, NF-KB, AND PLCG2-CA2+ SIGNALING AND PREVENTS INFLAMMATORY BONE LOSS

C. H. Lee¹, C. H. Chung¹, J. Y. Kim², Y. J. Choi³, W. H. Yoo³, M. S. Lee¹.

¹Wonkwang University Hospital, Internal Medicine, Iksan, Korea, Rep. of (South Korea); ²Wonkwang University, School of Medicine, Iksan, Korea, Rep. of (South Korea); ³Chonbuk National University Hospital, Internal Medicine, Jeonju, Korea, Rep. of (South Korea)

Background: Betulinic acid (BA), a natural plant-derived pentacyclic triterpenoid compound, is known to possess numerous pharmacological and biochemical properties including anti-inflammatory, anti-cancer, and anti-adipogenic activity.