Table. Risk of herpes zoster: Results of adjusted regression analyses with and without inverse probability weights

	Multivariate Analysis without IPW		Multivariate Analysis with IPW	
	Adjusted HR (95% CI)	P Value	Adjusted HR (95% CI)	P Value
Female sex	1.42 (1.12-1.82)	0.0042	1.21 (0.96-1.53)	0.1095
Age per 10 years	1.23 (1.13-1.33)	<.0001	1.31 (1.2-1.43)	<.0001
Glucocorticoids, 5-10 vs 0 mg/d	1.16 (0.95-1.41)	0.1577	1.23 (1-1.52)	0.0501
Glucocorticoids, >10 vs 0 mg/d	1.58 (1.02-2.46)	0.0417	1.92 (1.27-2.92)	0.0022
<i>csDMARD treatment</i>	<i>Reference</i>		<i>Reference</i>	
Monoclonal TNFi antibodies	1.55 (1.20-2.00)	0.0009	1.63 (1.25-2.12)	0.0003
Soluble TNF receptors	1.32 (0.98-1.77)	0.0683	1.34 (0.98-1.83)	0.0631
T-cell co-stimulation modulator	1.41 (0.97-2.05)	0.0746	1.69 (1.17-2.45)	0.0048
B-cell targeted therapies	1.45 (1.07-1.97)	0.0156	1.66 (1.19-2.3)	0.0026
IL-6 inhibitors	1.31 (0.97-1.77)	0.0737	1.55 (1.15-2.09)	0.0045
JAK inhibitors	3.55 (2.33-5.41)	<.0001	5.01 (3.45-7.28)	<.0001

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Preclinical models of arthritis and bone disease

OP0239

WHY DOES ALCOHOL INHIBIT ARTHRITIS? - AN EXPLANATION OF THE MECHANISM OF ARTHRITIS INHIBITION BY ETHANOL

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Background: Alcohol consumption has emerged as consistent protective factor for the development of autoimmune diseases such as rheumatoid arthritis (RA). The underlying mechanism for this tolerance-inducing effect of alcohol, however, is unknown.

Objectives: To understand the anti-arthritis effect of alcohol

Methods: The immune-regulatory properties of alcohol consumption in vivo were tested in the collagen-induced arthritis (CIA) and serum-induced arthritis (SIA) model as well as after immunization with T cell-dependent (NP-CGG) and independent (TNP-FICOLL) antigens. Additional experiments in vivo experiments in these models were done with acetate- the metabolite of ethanol. The models were analysed for T-cell lineage and plasma cell differentiation, germinal centre formation and IgG levels and sialylation. Molecular expression of T follicular helper cell (TFH) activation such as IL-21, Bcl-6 and PD-1, as well as TFH: B cell conjugates were also assessed. Furthermore, TFH cells were generated in vitro, exposed to ethanol or acetate and tested for IL-21 production, PD1 expression and conjugate formation with B cells.

Results: Ethanol exposure significantly inhibited arthritis in the active adaptive immunity-driven model of arthritis (CIA) but not in the passive innate immunity-driven model (STA) suggesting that the immune suppressive effect of alcohol is based on interference of T- and B- cell activation. In line ethanol and even more its metabolite acetate, suppressed T cell dependent antibody formation after NP-CGG immunization, while T cell independent antibody formation after TNP-FICOLL immunization was not suppressed. Ethanol, as well as its metabolite acetate, specifically altered the functional state of T follicular helper (TFH) cells in vitro and in vivo, thereby exerting immune regulatory and tolerance-inducing properties. Alcohol-exposed mice showed reduced Bcl6 and PD-1 expression as well as interleukin (IL)-21 production by TFH cells, preventing proper spatial organization of TFH cells to form TFH: B cell conjugates in the germinal centre. This effect of alcohol on TFH cells was associated with impaired autoantibody formation, higher sialylation of autoantibodies and less arthritis. In addition, overexpression of IL-21 in vivo completely reversed the immune regulatory effects of alcohol.

Conclusion: In summary, these data provide a new mechanistic explanation for the immune regulatory and tolerance-inducing effect of alcohol consumption in arthritis.

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OP0240

A MULTIMODAL MASS SPECTROMETRY APPROACH REVEALS SPECIFIC CARTILAGE MOLECULAR PROFILES ASSOCIATED TO TYPE 2 DIABETIC PATIENTS

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Background: Osteoarthritis (OA) is mainly characterized by the progressive deterioration of articular cartilage. Recent studies support that type 2 diabetes (TD2) is a risk factor to develop OA [1, 2]. However, the molecular cartilage profile of patients combining these two diseases remains unclear, and a better understanding of the different OA phenotypes should be considered for the development of personalized medicine.

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is used to investigate the bimolecular distribution of proteins, lipids or metabolites through the *in-situ* analysis of tissue sections. Bottom-up proteomics focuses on the relative quantification of proteins. The combination of both technologies could be considered to reveal specific molecular profiles and help for patient classification.

Objectives: The main goal of this study is to apply a multimodal mass spectrometry approach on cartilage to reveal specific lipidomic and proteomic profiles associated to TD2 patients.

Methods: Human cartilages from OA ($n_a=10$) and OA/TD2 human patients ($n_b=10$) were obtained from donors undergoing total knee joint replacement. Cartilage punches of 8*8mm were sectioned at 12 µm thickness for MALDI-MSI and bottom-up proteomics.

For MALDI-MSI experiments ($n_a=6$; $n_b=6$), norharmane matrix was sprayed over the samples for the detection of lipids. Experiments were then performed in positive ion polarity at 50 µm of lateral resolution using a RapifleX MALDI

Tissue-typing instrument. LipostarMSI and in-house ChemomeTricks toolbox for MATLAB software were used for data processing and analysis.

For bottom-up proteomics experiment ($n_a=10$; $n_b=10$), proteins were extracted, separated using SDS-PAGE and digested prior to liquid chromatography separation coupled to an Orbitrap MS Q-Exactive HF mass spectrometer. Proteome Discoverer, enrichR and Reactome software were used for data processing and analysis.

Results: MALDI-MSI showed overall differences between OA and OA/TD2 patients based on their specific lipidomic profiles. In particular, sphingomyelin and phosphatidylcholine species were significantly more abundant in OA patients whereas lysolipids such as lysophosphatidylcholine species were mainly present in OA/TD2 patients, providing therefore phenotype-specific OA molecular panels. Additionally, we observed that phosphatidylcholine and sphingomyelin species were more present in the superficial layer of the cartilage whereas lysophosphatidylcholine species were more abundant in the deep layer (Fig. 1A, B). Proteomics experiments applied on cartilage enables the quantification of 114 proteins. Among those, 73 were overexpressed in OA samples whereas 41 were overexpressed in OA/TD2 patients. Among the differentially regulated proteins (Fig. 1C), phospholipase A2 was increased in the diabetic cohort, in line with the elevated level of lysolipids found in the imaging data. Our results also involved the fatty acid omega oxidation and the fatty acid biosynthesis pathways as relevant to explain this deregulation of the lipid metabolism.

Conclusion: MALDI-MSI combined with proteomics experiments showed different profiles between OA and OA/TD2 patients and could be employed for patient classification.

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OP0241 IL9 MODULATION OF OSTEOCLASTS DIFFERENTIATION AND IMPACTS ON BONE METABOLISM

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Background: Osteoclasts are multinucleated cells originating from the monocytes/macrophage lineage and require receptor activator of NF- κ B ligand (RANK-L) and macrophage-colony-stimulating factor (M-CSF) for their development. They play a major role in bone remodeling by degrading the calcified bone matrix. They are considered as one of the culprits in bone destruction in many inflammatory diseases e.g. rheumatoid arthritis and osteoporosis. In previous work by our group, it was observed that IL-9 mediated the resolution of inflammation in rheumatoid arthritis and hence protected against bone degradation in animal models. Despite this protection was particularly associated to the resolution of inflammation, our data also supported the hypothesis of a direct signalling of IL-9 to osteoclasts.

Objectives: The aim of this study was to investigate the modulating effect of IL-9 on osteoclasts and on the bone metabolism.

Methods: Osteoclasts differentiation was studied in the mouse models of antigen induced arthritis (AIA) and KBXN serum induced arthritis (SIA) in the presence and absence of IL-9 by histomorphometric analysis and microcomputed tomography scans (μ CT). Osteoclasts were generated from bone marrow derived monocytes of BALB/c mice with M-CSF, RANKL and IL-9, which were added in varying concentrations to induce osteoclast differentiation. Tartrate-resistant acid phosphatase (TRAP) staining was performed to follow the differentiation steps from monocytes into multinucleated osteoclasts and to determine the effects of IL-9 on osteoclastogenesis. Additionally, we performed RNA-seq and seahorse analysis to detect IL-9 dependent, differentially expressed genes and metabolites. Intracellular signaling as induced by IL9R activation was followed by western blot analysis.

Results: IL-9 KO mice showed higher numbers of osteoclasts as compared to wild-type mice in the mouse models of AIA and SIA. Microcomputed tomography showed pronounced loss of the trabecular network and bone volume as signs of inflammation-induced osteopenia in IL9-/- mice. We found that osteoclasts express high levels of IL-9R. Next, monocytes were differentiated into osteoclasts in the presence of different concentrations of recombinant IL-9. Cells cultured in the presence of IL-9 showed significantly impaired differentiation into osteoclasts. We observed phosphorylation of STAT3 and STAT5 in cultured osteoclasts in dependency of IL-9. Furthermore, the presence of IL-9 during osteoclast differentiation impacted the gene expression levels of characteristic osteoclast related genes such as NFATc1, Cathepsin K and TRAP. Furthermore, IL-9 showed a major impact on mitochondrial respiration rate and glycolysis as assessed by Seahorse assays.

Conclusion: IL-9 exerted direct effects on osteoclast differentiation and modulated the expression of several genes that are related to osteoclast differentiation and function.

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OP0242 META-ANALYSIS OF SINGLE-CELL RNA SEQUENCING DATA OF THE SYNOVIUM TO DEFINE SYNOVIAL FIBROBLAST PHENOTYPES ACROSS JOINT LOCATION AND DISEASE

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Background: Up to now, three groups used single cell RNA sequencing (scRNA-seq) to analyse the synovium in arthritis using different methods and material to measure RNA expression on a single cell level: Ref. 1 used unsorted dissociated synovial cells and a droplet based method; Refs 2 and 3 performed scRNA-seq on sorted cell populations.

Objectives: The aim of this study was to perform a meta-analysis of scRNA-seq data of the synovium in arthritis: 1) to define synovial fibroblast (SF) phenotypes, 2) to confirm differences across SF clusters between rheumatoid arthritis (RA) and osteoarthritis (OA) and 3) to analyse joint specific differences between SF phenotypes.

Methods: In addition to the available count matrices [1-3], we used unsorted dissociated synovial cells from three patients with undifferentiated arthritis (UA) with a droplet-based method (10x Genomics). We followed a standard protocol [4] to integrate the datasets into a shared space, even in the presence of extensive technical and/or biological differences ("batch-corrected"). SF were selected as previously described (*PDPN*+, *ISLR*+, *COL1A2*+, *PTPRC*-) [1-3]. We used a minimum log2 FC of 0.25 for average expression of genes in a cluster relative to the average expression in all other clusters combined to define marker genes. R with Seurat, Monocle and clusterProfiler packages were used for scRNA-seq analysis, pseudotime trajectory analysis and pathway enrichment analysis, respectively. Quantitative PCR (qPCR) ($n=6-14$ per location and disease), immunohistochemistry (IHC) and Krenn synovitis score ($n=5-15$ per location and disease) were performed according to standard protocols.

Results: Data from 29 RA, 3 UA and 6 OA patients were analysed. From a total of 29'448 cells, we identified 14'787 (50%) with a fibroblast phenotype. Of those, we determined 5 subpopulations (Fig. 1): 1) *THY1*⁺ *CD55*^{hi} fibroblasts with high