-2, -3 and *-6* in peripheral blood mononuclear cells (PBMCs) in relation with *IL-23R* SNPs previously associated with AS.
Methods: We studied 74 patients (64,8% males) recruited from the Rheumatology Unit of the Puerta de Hierro Hospital diagnosed of AS following the Modified New York Criteria. The study cohort included patients with a mean age of 55.2±11, 2 years. Total RNA was extracted from PBMCs using the Nucleospin RNA kit (MN) and reverse transcribed into cDNA. mRNA expression was assessed by real-time quantitative RT-PCR using specific primers and Power SYBRGreen PCR Master Mix (Applied Biosystems).SNP genotyping [rs1129026 (G/A), rs10489629 (T/C), rs1343151 (G/A) rs2201841 (C/T), rs1004819 (C/T) y rs11209032 (A/G)] was performed using the Sequenom MassARRAY platform. In 17 cases there were two samples from the same patient. These samples were obtained from two scheduled visits and 99 samples were analyzed so. To determine the effect of independent variables on levels of *SOCS* genes expression, we fitted population-averaged models by generalized linear models, nested by patient, using the *xtgee* command of Stata v.12. *P*-values of <0.05 were considered statistically significant.
Results: Cellular *SOCS-1*,*-2* and *-6* expression did not show significant differences between the risk alleles carriers and the protective alleles carriers in any of the *IL-23R* SNP studied. *SOCS-3* increased significantly in protective alleles carriers of the *IL-23R* intronic SNP rs10489629-C (CC>CT>TT; *P*=0.028), the *IL-23R* non-synonymous SNP (Arg381Gln) rs11209026_A (AG>GG; *P*=0.047) and the *IL-23R* intronic SNP rs1343151-A (AA>AG>GG, *P*=0.005).
Conclusions: Higher *SOCS-3* expression levels for AS patients carriers of protective alleles of the *IL-23R* rs10489629-A, rs11209026-A and rs1343151-A as compared to carriers of risk genotypes could influence the pathogenesis of this disease.
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FRI0421 DYSREGULATED MIR-125 PROMOTES JOINT ANGIOGENESIS IN PSA THROUGH ALTERED BIOENERGETICS

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Background: Psoriatic arthritis (PsA) is characterised by an early vascular phase which is essential in perpetuating pannus growth, immune responses and disease progression. Recently, numerous studies have highlighted the emerging importance of endothelial cell metabolism in controlling angiogenesis. Herein, we propose microRNA, miR-125, modulates EC bioenergetics and orchestrates joint angiogenesis as characterised using *ex-vivo and in-vitro* tissue/cell assays and a novel *in-vivo* zebrafish model.
Objectives: To examine the relationship between miR-125, angiogenesis and cellular metabolism in the PsA synovium.
Methods: Primary PsA synovial fibroblasts (PsA FLS) and microvascular endothelial cells (HMVEC) were transfected with anti-miR-125a. Angiogenic mechanisms were quantified using tube formation assays, invasion by Transwell Matrigel chambers, migration by wound repair and metabolic gene expression by RT-PCR. Real-time analysis of extracellular acidification rates (ECAR) and oxygen consumption rates (OCR) of anti-125 treated HMVEC was assessed using the XF-24 Flux Analyzer (Seahorse Bioscience). To determine if altered metabolism is observed *ex vivo*, glycolysis/oxidative phosphorylation markers (GAPDH/PKM2/GLUT1/ATP), and angiogenic factors (FactorVIII/VEGF/ANG2) were quantified by immunohistology. MiRNA levels were quantified in synovial tissue (ST) and PBMC by RT-PCR and compared to clinical marker and immunohistochemical analysis. The angiogenic effects of miR-125 were assessed *in vivo* using GFP-tagged zebrafish embryos treated with anti-125 morpholinos, or vitamin D3 analog, calcitrol, to monitor vascular development.
Results: An increase in tube formation, cellular invasion and/or migration mechanisms ($p<0.05$) were demonstrated in anti-miR-125a transfected HMVEC and FLS supernatant. Inhibition of miR-125 significantly decreased basal, maximal and spare respiratory capacity ($P<0.009$) with a concurrent decrease in ATP synthesis ($P<0.008$). Increased glycolysis was further confirmed by the observed elevation of glycolytic genes: HK2, GSK3A, PDK1, HP6G (ns) and 3PO target and stimulator of glycolysis, PFKFB3 ($P<0.05$). *In vivo* synovial expression of miR-125 was significantly decreased in PsA versus OA synovial tissue and was associated with increased PsA macroscopic and microscopic vascularity. This was paralleled by a significant increase in the vascular expression of glycolytic markers, PKM2, GLUT1 and ATP5B in PsA compared to OA synovium. Finally, anti-125 morpholinos treated Zebrafish displayed increased vascular sprouting. In contrast, Calcitriol significantly reduced vascular development and increased the expression of miR-125, promoting miR-125 as a potential mechanism for orchestrating angiogenic development *in vivo* and in response to pharmaceutical agents
Conclusions: Our data demonstrates decreased expression of miR-125 in PsA synovium and *in-vivo* models was strongly associated pro-angiogenic mechanisms. Elevated glycolysis following miR-125 inhibition may enables endothelial cells to meet the increased energy and biosynthetic demands for new vessel formation. Correcting these deficiencies and their resulting metabolic shift, either by conventional pharmacological or as novel drug targets, may provide therapeutic benefit, especially in early disease.
Disclosure of Interest: None declared
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FRI0422 ESTROGEN ATTENUATES THE DISEASE ACTIVITY OF SPONDYLOARTHRITIS IN SKG MICE

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Background: Ankylosing spondylitis is a male-predominant disease, and the male gender is also associated with more severe radiographic damage. Estrogen modulates immune-related processes such as T cell differentiation and cytokine production.
Objectives: This study aimed to evaluate the role of estrogen in the disease activity of spondyloarthritis (SpA). The effect of estrogen on the inflammatory cytokines was evaluated.
Methods: The effects of estrogen on the development of arthritis were evaluated by performing an ovariectomy and E2 pellet implantation in the zymosan-treated SKG mouse. Clinical arthritis scores were measured and PET-CT was performed to quantify joint inflammation. Total RNA was extracted from the hindpaws and forepaws and the expression of TNFα, IL-6, IFNγ, IL-4, IL-17A, IL-23, Dkk1, and SOST was measured by QuantiGene 2.0 plex assay.
Results: Zymosan exposure triggered SpA-like diseases in SKG mice, including peripheral arthritis, spondylitis, dactylitis, enteritis, and psoriatic skin lesions. E2-treated mice showed remarkable suppression of arthritis clinically and little infiltration of inflammatory cells in the Achilles tendon and intervertebral disc. ^{18}F-FDG uptake was significantly lower in the E2-treated mice. The expression of TNFα, IFNγ, IL-17A, Dkk1, and SOST was significantly reduced in E2-treated mice compared with sham and ovariectomized mice.
Conclusions: Estrogen suppressed arthritis development in SpA model of SKG mouse. Results of the study suggest that estrogen may have an anti-inflammatory effect on the disease activity of SpA.
Disclosure of Interest: None declared
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FRI0423 ANTIBODIES TO TYPE II COLLAGEN: A NOVEL TOOL FOR THE SPONDYLOARTHRITIS DIAGNOSIS?

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Background: Spondyloarthritis (SpA) are an inflammatory joint disease with chronic, progressive, axial inflammation of the spine and the sacroiliac joints. Diagnosis of SpA is done criteria by clinical symptoms, radiology and MRI or ultrasound following ASAS criteria. AS is similar to rheumatoid arthritis (RA) and psoriatic arthritis (PsA) as they are all inflammatory joint disease. Nevertheless they show considerable different pathology. [1]
Objectives: The aim of our study is to test whether a novel assay that we developed for RA can be used for SpA diagnosis. We have previously showed that antibodies to oxidative post-translationally modified collagen type II (oxPTM-CII) are present specifically in RA patients whether ACPA positive or negative. [2]
Our study intends to investigate the reactivity to oxPTM-CII in SpA patients in comparison to early undifferentiated arthritis (EUA) and PsA patients.
Methods: oxPTM-CII were generated using ribose and various reactive oxidants, and then they were analysed by SDS-PAGE. Binding to native and oxPTM-CII was evaluated by ELISA and Western Blotting. We used a cohort of sera from 67 patients with SpA, 54 patients with PsA, 49 patients with EUA. As control we used 19 patients with fibromyalgia (FM) and 70 healthy subjects. The specificity of the binding was further assessed by competitive ELISA and western blot.
Results: We detected stronger reactivity to SpA compared to PsA and even EUA serum samples. Hence specific binding to oxPTM-CII was seen in the 52% of SpA sera compared to 12% in PsA and 10% in EUA. There was no binding in samples from FM and healthy individuals. A group of the most reactive SpA samples was evaluated by western blot confirming a strong binding to several fragments or aggregates of oxPTM-CII.
Conclusions: For the first time we demonstrated that anti-ROS-CII may become a biomarker for SpA diagnosis.
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FRI0424 EQUAL PRESENCE OF CIRCULATING MAIT CELLS IN AXIAL SPA PATIENTS WITH ONLY AXIAL INVOLVEMENT AND AGE- AND SEX-MATCHED HEALTHY CONTROLS

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Background: Previous studies indicated a potential role for mucosal-associated invariant T (MAIT) cells in the pathogenesis of ankylosing spondylitis (AS).[1,2] Active peripheral arthritis and extra-articular manifestations may influence the presence of circulating MAIT cells in AS.
Objectives: To investigate circulating MAIT cells in a homogenous group of axial spondyloarthritis (SpA) patients with only axial involvement in comparison to age- and sex-matched healthy controls (HC). Secondly, to explore the association of MAIT cells with symptom duration and disease activity.
Methods: Consecutive axial SpA patients from the Groningen Leeuwarden axial SpA (GLAS) cohort without active peripheral arthritis, inflammatory bowel disease, psoriasis or uveitis were included. Patients with active infections or current use of biologics were excluded to rule out possible influence on the presence of circulating MAIT cells. Disease activity was assessed using ASDAS, BASDAI, and serum CRP levels.
The frequencies and absolute numbers of circulating MAIT cells were examined in peripheral blood of all studied samples by 5-color flow cytometry. Immediately after sampling, EDTA-blood was stained with anti-CD3, anti-CD8, anti-TCRVa7.2, anti-CD161, and anti-TCRgd. After staining, the cells were washed, fixed, and analyzed immediately on FACS. MAIT cells were identified phenotypically as $CD3^+CD8^+TCR\gamma\delta^-V\alpha7.2^+CD161^{high}$ cells.
Results: Of the 41 included axial SpA patients, mean age was 46±16 years, 73% were male, mean symptom duration was 23±14 years, and 78% were HLA-B27 positive. Mean ASDAS was 2.6±1.0, mean BASDAI was 4.4±2.5, and median CRP was 3 (range 2–30). 70%, 54% and 37% of axial SpA patients had ASDAS $\geq$2.1, BASDAI$\geq$4 or CRP$\geq$5, respectively. HC had exactly the same age and sex distribution.
Both the percentages and absolute numbers of circulating MAIT cells were comparable between axial SpA patients and HC (Figure 1). In axial SpA patients, absolute numbers of MAIT cells correlated negatively (rho=-0.339) with symptom duration. No significant associations were found between MAIT cells and disease activity, except for a negative correlation (rho=-0.332) between frequency of MAIT cells and BASDAI (Table 1). There were no significant differences in MAIT cells between axial SpA patients with and without active disease according to ASDAS, BASDAI or CRP.

Table 1. Association of percentages and absolute numbers of MAIT cells with symptom duration and assessments of disease activity in axial SpA patients (n=41), Spearman correlation coefficients

	% CD8+ MAIT	nr CD8+ MAIT
Symptom duration (yrs)	-0.248	-0.339*
$ASDAS_{CRP}$	-0.221	0.053
BASDAI (0–10)	-0.332*	-0.126
CRP (mg/l)	-0.137	0.030

*P-value <0.05.

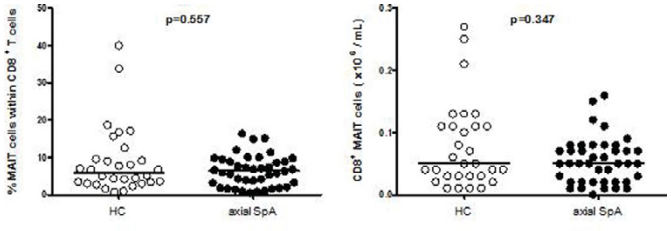

Figure 1. Percentages and absolute numbers of circulating CD8+ MAIT cells in axial SpA patients (n=41) and matched healthy controls (n=30), Mann-Whitney U tests.

Conclusions: In this homogeneous group of axial SpA patients with only axial disease, the presence of circulating MAIT cells did not differ from age- and sex-matched HC. No strong association was found between circulating MAIT cells and symptom duration or disease activity.
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FRI0425 ANALYSIS OF CELLULAR COMPOSITION AND CYTOKINE EXPRESSION IN THE SUBCHONDRAL BONE MARROW IN ANKYLOSING SPONDYLITIS

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Background: Ankylosing spondylitis (AS) is characterized by inflammation within the sacroiliac joints and at the spine including vertebral bodies and facet joints. In AS, bone destruction is followed by new bone formation leading to ankylosis of joints and syndesmophyte development. Histological studies of bone material from AS patients showed subchondral bone marrow changes, namely the transition of the bone marrow into granulation tissue which facilitates subchondral bone destruction but also promotes local bone formation [1, 2]. Currently, it is unclear what promotes the transition of the bone marrow into the granulation tissue.
Objectives: The aim of this study was to look for changes in the subchondral bone marrow in joints from AS patients that may precede and promote transformation into granulation tissue. Therefore, we analyzed the cellular composition of the bone marrow and determined local cytokine expression at subchondral regions of AS facet joints.
Methods: Facet joints were acquired from AS patients undergoing polysegmental correction surgery and compared to joints from autopsy controls. We performed immunohistochemical stainings to determine the number of T cells (CD3) and B cells (CD20), macrophages (CD68, CD163), monocytes (CD14), dendritic cells (DC; CD1a, DCsign) and myelopoietic cells (MPO). To correct for putative differences in fat cell content, software assisted image analysis was used to calculate the area of bone marrow covered by fat cells and the number of nucleated cells (DAPI) as well as myeloid cells. Cytokine expression was determined by mRNA in situ hybridization (TNF, IFNg) or immunohistochemistry (TGFβ, IL-10).
Results: We observed no difference in the number of adaptive immune cells, i.e. CD3+ T and CD20+ B cells, between AS and control joints and found no difference in T/B cell aggregates. Furthermore, no difference in the number of macrophages (CD163+, CD68+) and DCs (CD1a+, DCsign+) was found. The only difference in cellular composition appeared to be a slight trend towards a higher percentage of myelopoietic MPO+ cells/DAPI+ cells in AS joints compared to control joints. The overall number of DAPI+ cells as well as the fat content of the bone marrow was not different between AS and control joints.
Concerning cytokines, TNF mRNA expression was in general low but significantly increased ($p<0.05$) in AS versus control joints. No difference was found for IFNg. The number of TGFβ expressing cells was similar in AS and control joints while the number of IL-10 positive cells was decreased ($p<0.01$) in AS joints.
Conclusions: The results show a shift from pro-inflammatory to anti-inflammatory cytokine expression within subchondral bone marrow sites in AS joints while no major change in the cellular distribution of the major leukocyte subsets is found. The shift in cytokine milieu may contribute to transformation of the bone marrow into granulation tissue. It would be interesting to determine the cellular source of local cytokines, such as of IL-10.
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FRI0426 NO DEMONSTRABLE EFFECT OF IL-23 RECEPTOR VARIANTS ON CLINICAL MEASURES AND IL23/ IL-17 LEVELS IN ANKYLOSING SPONDYLITIS

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Background: IL23 binding to IL23 receptor (IL23R) is necessary for the maturation of Th17 cells and generation of proinflammatory IL17 and TNF in AS. IL23R variants have considerable impact on AS susceptibility in genome-wide association studies.
Objectives: To describe the effect of genotypic IL23R variants on proinflammatory cytokines and disease measures in AS.
Methods: Cross sectional cohort study of patients with established AS (n=334, 90% B27 +, mean age at study 45 years) included in a disease registry. IL23R genotyping for nonsynonymous SNP's (rs11209026 (protective allele A) and rs11209032 (risk allele A) was done by Taqman RT-PCR, while IL23, IL17, IL6 and TNF levels determined by sandwich ELISA and compared with age and gender matched healthy controls (n=72). Genotypic associations with clinical and serological features were analyzed with nonparametric methods.
Results: There was no significant difference between in AS patients and controls