

Hg<sup>1</sup>). For transcriptome analysis, total RNAs from whole peripheral blood cells were extracted with using PAXgene miRNA kit. After constructing single-stranded, strand-specific libraries, multiplex sequencing was done. After quantifying the expressions of transcripts, differentially expressed genes (DEGs) between exPH and exN group were selected by paired T-test ( $P < 0.05$ ). And then, hierarchical clustering analysis and pathway enrichment analysis (PathVisio) were performed.

**Results:** There were no significant differences between exPH and exN group in the result of total skin score, serum BNP, tests of pulmonary function and thermography after 0°C-stress. Positive SSc-related autoantibody was a risk factor for exPH (odds ratio, 1.41); especially, positive anti-RNP seemed to be prominent (odds ratio, 3.21). Based on the 817 DEGs between exPH and exN group, the hierarchical clustering showed major 4 clusters, and one of them consisted of only cases in exPH group. When we focused on 117 genes reported to be directly implicated in the development of PAH<sup>2</sup>), it is noteworthy that 4 of them including TGF- $\beta$  induced protein were differentially expressed. Pathway analysis of transcriptome revealed that 22 pathways, such as hypertrophy model, lung fibrosis and Wnt/B-catenin signaling, were differently enriched between exPH and exN group.

**Conclusions:** The paradigm of SSc-PAH management should ideally be aimed at detecting early PVD and starting treatment prior to fulfilling the criteria for PAH. Although detection of early PVD in SSc patients remains a major challenge, exercise DE seemed to be a good, non-invasive method for screening. It is noteworthy that expression changes in some of known PAH-related genes were detected from peripheral blood of exPH patients. It shows the possibility that the therapeutic intervention at early stage of the disease may alter the clinical course.

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#### FRI0418 FACTORS ASSOCIATED WITH STEROID-FREE REMISSION IN PATIENTS WITH INFLAMMATORY MYOPATHIES. A RETROSPECTIVE ANALYSIS OF A SINGLE-CENTER COHORT

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**Background:** The inflammatory myopathies are a heterogeneous group of connective tissue diseases characterized by muscle weakness and inflammation. Corticosteroids are the standard main treatment for inflammatory myopathies. However, steroid therapy often causes a wide range of side effects. Although immunosuppressive drugs are used as steroid-sparing agents in an effort to prevent disease recurrence, the appropriate duration of steroid use remains unclear.

**Objectives:** We investigated whether steroid therapy can be safely withdrawn in patients with inflammatory myopathies followed in a single center.

**Methods:** We retrospectively reviewed clinical charts of 71 consecutive patients (age 51.9 $\pm$ 15.7 y.o., female 69%) who met Bohan and Peter criteria for polymyositis (PM)/dermatomyositis (DM) and modified Sontheimer's criteria for clinically amyopathic dermatomyositis (ADM), respectively. Steroid free remission was defined as a 3-month consecutive period of no disease activity without corticosteroid treatment. Factors associated with steroid free remission were examined.

**Results:** Of 71 identified patients, 29 patients (40%) were DM, 15 patients (21%) were PM, 9 patients (13%) were overlap myositis, and 18 patients (25%) were ADM. Thirty-seven patients (52%) had muscle weakness, 5 patients (7%) had malignancies and 43 patients (61%) had signs of interstitial lung disease. With a mean follow-up of 6.6 $\pm$ 5.0 years, 9 patients (13%) died during follow-up period. The remaining 62 patients were treated with corticosteroids alone or in combination with immunosuppressants. Steroid-free remission was achieved in 21 of 62 patients (34%) patients with a mean time to steroid withdrawal of 5.5 $\pm$ 4.0 years. Six of 21 patients (29%) relapsed 1.7 $\pm$ 1.7 years after steroid withdrawal. There were no differences in onset of age, disease duration, positive ANA, positive Anti Jo-1 antibodies, serum creatine kinase levels, maximum dose of corticosteroids, skin, joint and lung involvement between steroid-free group and non-steroid-free group. Elevated inflammatory markers were associated with long-term steroid use ( $p=0.038$ ). Concomitant immunosuppressants were more frequently used in non-steroid-free group than steroid-free group ( $p=0.002$ ).

**Conclusions:** Steroid-free remission might be achieved in some patients with inflammatory myopathies.

**Disclosure of Interest:** None declared

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#### FRI0419 THE PREDICTIVE PROGNOSTIC FACTORS FOR CLINICAL COURSE OF POLYMYOSITIS/DERMATOMYOSITIS-ASSOCIATED INTERSTITIAL LUNG DISEASE

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**Background:** Interstitial lung disease (ILD) and concomitant infectious diseases are the predominant causes of death in polymyositis/dermatomyositis (PM/DM). We have already reported that hypoxapnea and ILD lesion in upper lung fields are independent prognostic factors. Micro RNA is a non-coding RNA, which has a certain function such as transcriptional regulation. miR-1 has been reported to be associated with myocyte differentiation and to decrease in muscle tissue from patients with inflammatory myopathies.

**Objectives:** Here we investigated the association of serum miR-1 level with clinical course of PM/DM-associated ILD (PM/DM-ILD).

**Methods:** We retrospectively analyzed clinical baseline, serum miR-1 level, initial therapeutic regimens, total amounts of PSL, clinical outcomes, and episode of infection of patient with PM/DM-ILD who had received initial treatment at six hospitals associated with Yokohama City University from 2003 to 2016. The serum miR-1 level was measured by quantitative real-time PCR.

**Results:** One hundred sixteen (PM 22, DM 51, and clinically amyopathic DM 43) patients were included. The mean age was 56 $\pm$ 15 years and 83 were female. As initial therapies, oral PSL, methylprednisolone (mPSL) pulse, intravenous cyclophosphamide (IVCY), and oral calcineurin inhibitor therapies were performed in 113 (97%), 80 (69%), 48 (41%) and 80 (69%), respectively. Forty-one patients had a serious infection at 51 $\pm$ 38 days from initiation of immunosuppressants and 10 died of infections. Old age, low PaCO<sub>2</sub> and albumin, high LDH and KL-6, high score of ILD, high initial dose of PSL, mPSL pulse, IVCY, calcineurin inhibitor and combination therapy were extracted as risk factors for infection by univariate analyses. A multivariate logistic regression analyses revealed that combination therapy ( $p=0.012$ , OR 2.83), old age ( $p=0.024$ , OR 2.12), high initial dose of PSL ( $p=0.024$ , OR 2.69), low albumin ( $p=0.031$ , OR 3.56), and low PaCO<sub>2</sub> ( $p=0.038$ , OR 2.67) were independent risk factors for infection. Serum samples were obtained from total of 14 patients and 13 healthy controls. Serum miR-1 levels in PM/DM-ILD patients before treatment were significantly higher than those in healthy controls ( $p=0.047$ ). Also serum miR-1 levels were significantly higher in PM/DM-ILD patients with concomitant infectious diseases as compared to patients without infectious diseases ( $p=0.043$ ). We further divided the PM/DM-ILD cases into two groups by the serum miR-1 level. The higher miR-1 group showed poorer effectiveness of ILD treatment ( $p=0.040$ ), and lower lymphocyte count ( $p=0.013$ ) as compared to the lower miR-1 group.

**Conclusions:** Appropriate monitoring is important for PM/DM-ILD, especially in older patients with malnutrition or decreased respiratory function. miR-1 can be a new biomarker for predicting treatment response and concomitant infectious diseases during treatment for PM/DM-ILD.

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#### Spondyloarthritis - etiology, pathogenesis and animal models

#### FRI0420 ASSOCIATION OF SUPPRESSOR OF CYTOKINE SIGNALING -3 (SOCS-3) EXPRESSION WITH INTERLEUKIN-23 RECEPTOR (IL-23R) SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN ANKYLOSING SPONDYLITIS (AS)

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**Background:** Nowadays genetic-association studies have discovered new genes, other than HLA-B27, as IL-23R associated with AS. The signalling pathway through IL-23R is negatively regulated by the SOCS proteins. However, the reports regarding the roles of SOCS in AS are very rare at present.<sup>1,2</sup>

**Objectives:** The aim of this study is to assess the gene expression of SOCS-1,

-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, calcitriol, 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 TNF $\alpha$ , IL-6, IFN $\gamma$ , 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. <sup>18</sup>F-FDG uptake was significantly lower in the E2-treated mice. The expression of TNF $\alpha$ , IFN $\gamma$ , 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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