Background: Fibrotic changes in the myocardium and cardiac arrhythmias represent fatal complications in systemic sclerosis (SSc). So far, the underlying mechanisms remain elusive. Mice overexpressing AP-1 family transcription factor Fosl-2 (Fosl-2tg) resemble animal model of SSc.

Objectives: We aimed to identify the mechanisms controlling myocardial fibrosis and arrhythmias in SSc.

Methods: Mice overexpressing activator protein-1 transcription factor component Fos-related antigen-2 (Fosl-2tg) and Rag2−/−Fosl-2tg mice lacking T and B cells were subjected to study myocardial fibrosis, arrhythmias and response to stress using microelectrode, echocardiography and ex vivo Langendorf-system. Immunohistochemistry/immunofluorescence analyses were used to characterize endomyocardial biopsies (EMBs) of SSc patients with heart failure with or without arrhythmia, and mouse hearts. Cardiac human and mouse fibroblasts were used to evaluate molecular mechanisms using bulk RNA sequencing, qPCR, IF, ELISA, Western blot, Bromodeoxyuridine proliferation assay, senescence associated-1-Galactosidase assay for cell senescence, Caspase Glo 3/7 assay for cell apoptosis and contraction assay.

Results: Fosl-2tg mice showed interstitial cardiac fibrosis with increased numbers of CD45+CD31+Ter119+gp38+ cardiac fibroblasts, disorganized connexin 43 and 40 in intercalated discs and deregulated expression of genes controlling conduction system (Mef1, Nfkbeta, Tbx3, Sod5, Scr1, Kcnq1, Scn1a, Nop5, Tap). Fosl-2tg mice developed higher heart rate (HR), prolonged QT intervals, arrhythmias with prevalence of premature ventricular contractions, ventricular tachycardias and atrio-ventricular blocks second-degree. QT intervals positively correlated with AV blocks, while QT intervals and AV blocks positively correlated with the disease phenotype and collagen deposition. Fosl-2tg mice showed significantly reduced HR variability in long-term ECG recordings (reduced NN intervals, SDNN, RMSSD, NN50, pNN50), indicating autonomic imbalance with a shift towards increased sympathetic activity. Following stimulation with isoproterenol (ISO) Fosl-2tg mice revealed impaired HR response. Similarly, ex vivo Langendorff-system measurement of HR in the isometric Fos-related antigen-2 (Fosl-2tg) and Rag2−/−Fosl-2tg/Fosl-2tg mice lacking T and B cells revealed that systemic inflammation triggered fibrotic changes in the myocardium and cardiac arrhythmias indicative of autonomic disbalance with a shift towards increased sympathetic activity. To validate the predictions obtained, the effect of drug candidates already used in humans was tested in vitro and in vivo preclinical models: in 1) cultured human muscle cells treated with IFN-γ and in 2) a mouse model of myositis experimentally induced by immunization against skeletal muscle fast-type C protein.

Conclusion: These results demonstrate that under inflammatory and fibrotic conditions Fosl-2 drives myocardial fibrosis, arrhythmias and causes aberrant response to stress. This mechanism might represent a promising target against increased cardiac disease burden in the immunofibrotic cardiac disorders.

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OP0108 INTEGRATED ANALYSIS OF SINGLE-CELL AND BULK RNA SEQUENCING DATA REVEALS CELLULAR HETEROGENEITY IN THE SKELETAL MUSCLE OF IDIOPATHIC INFLAMMATORY MYOPATHY

Keywords: Cell biology, Myositis

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Background: IIMs-affected muscles showed diverse cellular microenvironments, and specific immune activation is involved in the different types of IIMs. Skeletal muscle cells exhibit immunobiological properties and act as nonprofessional antigen-presenting cells in IIMs.

Objectives: We sought to identify the skeletal muscle cells (SMCs) and immune cell populations in IIMs muscles and understand how alterations in these cell subpopulations lead to muscle damage.

Methods: We performed single-cell RNA-sequencing (scRNA-seq) on muscle tissues from 6 IIMs and 3 normal control (NC), and spatial transcriptomics from 2 IIMs and 1 NC. 76 IIMs and 10 NC were analysed by bulk RNA sequencing.

Results: From the scRNA-seq analysis, remarkable heterogeneity of SMCs and immune cell phenotypes were found and spatially organized in IIMs subgroups. They displayed diverse functions and cell-surface proteins. From the
bulk RNA-seq deconvolution analysis, cell clusters with high expression of type 1 interferon-related genes, such as IG5+ SMCs, CD4+IG15+ T cells, FCN1+IG15+ macrophages and KLRC1+XCL1+ NK cells, were specifically cloned in DM. Cell subpopulations involved in mitochondrial changes, such as Mitohigh CD8+ TEFs, were expanded in anti-Jo1 assay group. TPM1+ SMCs and Mitohigh NKs have the highest proportion in anti-SRF IMM. Mitohigh NKs were positively correlated with serum creatine kinase (CK) in IM patients. scRNA-seq and spatial transcriptomics combined analysis indicated ligand-receptor pairs MIF-CD74/CD44/MIF-CD74/CXCR4 play key roles in the interactions of ANKRD2+ SMCs and inflammatory cells. The levels of CD44 and CXCR4 in muscle tissues were positively correlated with CK, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase.

**Conclusion:** Our results demonstrate previously unrecognized SMCs and immune cells phenotypes in IIM subgroups, and indicate the interactions of SMCs and immune cells are key pathological mechanisms driving muscle damage.

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**OP0119**

**MUSCLE FIBRE PLAYS A CRUCIAL ROLE IN THE THERAPEUTIC RESPONSE OF MYOSITIS TO GLUCOCORTICOIDS THROUGH THE PARACRINE EFFECT OF EPINEPHRINE ON THE IMMUNE SYSTEM**

**Keywords:** Animal Models, Myositis

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**Background:** Glucocorticoids (GC) are the first line treatment in myositis. GC treatment is empirical. Both GC therapeutic and iatrogenic effects are mediated by the glucocorticoid receptor (GR), which is ubiquitously expressed. Our team has recently shown that muscle fibres immuno-metabolic modifications participate to muscle weakness and perpetuation of the disease[1]. Thus, myofibers could be a therapeutic target of GC.

**Objectives:** To unravel the mechanism of GC therapeutic effect in order to optimize myositis care.

**Methods:** Experimental myositis (EM) was induced in 8 to 10 week-old C57BL/6J mice through the immunization against a polypeptide from skeletal muscle fast-type C protein. In order to investigate whether GC target skeletal muscle fibres to elicit their therapeutic response in EM, we generated mice in which GR can be selectively ablated in skeletal muscle fibres in a temporal manner. To this end, EM was induced at day (D) 0 in HSA-Cre-ERT2(tg/0)/GRL2/L2 mice (pre-mutant mice), which express the tamoxifen-dependent CreERT2 recombinase selectively in skeletal muscle fibres and bear two LoxP-flanked GR alleles, as well as in HSA-Cre-ERT2(tg/+)GRL2/L2 littermates, which do not express the recombinase (GRL2/L2 mice). Thus, tamoxifen treatment from D9 to D13 induced GR inactivation in the former mice only, termed hereafter GR(i)skm−/− mice. Mice of both lines were treated by prednisone (PDN) from D14 to D20 at the dose of 1 mg/kg/day by gavage. Grip test was performed at D0, before the 1st PDN administration (D14) and the day before sacrifice (D20). Creatine-kinase (CK) activity assay in serum, muscle histology, immune-cell phenotyping using flow cytometry and gastrocnemius transcriptomic analysis were run. Transcriptomic data were validated in independent mice cohorts, in vitro and on human muscle biopsies.

**Results:** In pre-mutant EM mice at D0 as well as in EM GR(i)skm−/− at D14, muscle strength was still comparable to that of EM GRL2/L2 mice. At D20, muscle strength was still comparable between V-treated EM GRL2/L2 and GR(i)skm−/− mice. Conversely, PDN treatment did not induce a regain of muscle strength in EM GR(i)skm−/− mice (143.9 vs 175.7 ± 7.5 in PDN-treated GRL2/L2, p=0.0007). V-treated EM GRL2/L2 and GR(i)skm−/− mice showed a similar increase in serum CK levels at sacrifice. CK decreased only in GRL2/L2 and not in GR(i)skm−/− mice after PDN treatment (146.6 ± 10.9 vs 234.5 ± 27.3 p=0.002). Although no major differences in the histological inflammatory infiltrate score among the four experimental groups, at muscle flow cytometry, the percentage of proinflammatory macrophages, F4/80-Ly6c-positive, was greater (76% vs 67%, p=0.5) and that of anti-inflammatory macrophages, F4/80-CD206-positive, was lower (12% vs 17%, p=0.7) in PDN-treated GR(i)skm−/− than GRL2/L2 mice suggesting myofiber GR knockout promotes a proinflammatory phenotype in immune cells infiltrate. Strikingly, PDN induced a 3-fold decrease in the percentage of CD8-positive T cells in EM GRL2/L2 mice compared to V-treated EM GRL2/L2 mice (11% vs 29%, p=0.003). This anti-inflammatory effect of PDN was suppressed in PDN-treated EM GR(i)skm−/− mice. Moreover, CD4-CD8 double negative T cells, that inhibit the immune response by killer effector T cells, slightly increased in PDN-treated EM GRL2/L2 compared to V-treated GR L2/L2 mice (35% vs 24%, p=0.2). This effect of PDN was suppressed in PDN-treated EM GR(i)skm−/− mice. Transcriptomic and functional analyses of muscle in vitro and in vivo demonstrated the importance of epinephrine secreted by the myofiber relaying the effects of GC. The expression of epinephrine by the myofibre in response to GC has been validated in patients with myositis.

**Conclusion:** Skeletal muscle fibres play a critical role in the GC therapeutic response in myositis through an epinephrine-mediated polarization of inflammatory infiltrate toward an anti-inflammatory phenotype.


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**Predictors of outcome in early rheumatoid arthritis**

**Keywords:** Diagnostic Tests, Rheumatoid arthritis, Autoantibodies

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**Background:** Individuals testing positive for anti-cyclic-citrullinated-peptide-antibodies (Anti-CCP) and musculoskeletal (MSK) complaints are at risk for developing rheumatoid arthritis (RA).

**Objectives:** To identify factors involved in arthritis progression in a population considered at risk for RA.

**Methods:** Anti-CCP-positive individuals with MSK complaints referred to rheumatology in the Region Stockholm were recruited. Individuals lacked arthritis at clinical and ultrasound examination and were followed for ≥3 years or until arthritis diagnosis was made. Blood samples from inclusion were analyzed for 9 selected anti-citrullinated-protein-antibody (ACPA) reactivities (citrullinated α-1- enolase, fibrinogen, floggin, histone, vimentin and tenascin peptides); as well as a panel of 92 inflammation-associated proteins and HLA-SE alleles. Cox regression was applied to the data and a predictive multivariate model was identified. Results are shown with a confidence interval (CI) of 95 percent.

**Results:** 267 individuals were recruited. 101 (38%) developed arthritis in median after 14 months (IQR: 6-27). In the multivariate analysis: ACPA reactivity (HR 8.0, CI 2.9-22, p<0.0001), IL15R-α levels (HR 0.6, CI 0.4-0.9, p 0.006), IL6 levels (HR 1.5, CI 1.2-1.8, p<0.001) and the presence of tenosynovitis as detected by ultrasound (HR 3.4, CI 2.0-6.0, p<0.0001) were significantly associated with arthritis. Diagnostic accuracy for ACPA reactivity test had a sensitivity of 96% (CI 92-99.8), a specificity of 30% (CI 30-46), a positive predictive value of 51% (CI 43-58) and a negative predictive value of 94% (CI 87-99.7). Diagnostic accuracy for ultrasound assessed tenosynovitis had a sensitivity of 17% (CI 9-24), a specificity of 99% (CI 97-100), a positive predictive value of 89% (CI 74-100) and a negative predictive value of 64% (CI 57-70).

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