the "Slow Progressor" (3303 ± 6065 pg/ml vs 13300 ± 10308 pg/ml; $p=0.052$) without reaching a significant value, due probably to the low number of cases.

Conclusion: Our study confirms that the presence of specific autoantibodies and capillaroscopic abnormalities correlate to an increased risk of developing SSc in patients with UCTD [6]. Beside we found significantly higher levels of CXCL4 in our 27 VEDOSS patients respect to controls, in agreement with other authors showing the association of this chemokine with early stages and specific organ involvement [4][5][7]. The finding of CXCL4 lower levels in "fast progressor" cases is consistent with our recent report of anti-CXCL4 antibodies in patients with early SSc, determining lower levels of this antigen [7]. We need deeper investigations to better evaluate the role of CXCL4 in the different stages of SSc.

REFERENCES:

- [1] J. Avouac et al., Ann. Rheum. Dis., 2011.
- [2] F. van den Hoogen et al., Ann. Rheum. Dis., 2013.
- [3] G. Valentini et al., Clin. Exp. Med., 2017.
- [4] R. Lande et al., Nat. Commun., 2019.
- [5] L. van Bon et al., N. Engl. J. Med., 2014.
- [6] M. Vasile et al., Clin. Exp. Rheumatol., 2018.
- [7] R. Lande et al., Int. J. Mol. Sci., 2020.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2188

OP0247

IN MYOSITIS MUSCLE FIBRE PLAYS A DIRECT AND CRITICAL ROLE IN THERAPEUTIC RESPONSE TO GLUCOCORTICIDS

M. Giannini¹, G. Laverny², D. Duteil², D. Rovito², A. L. Charles³, B. Geny⁴, D. Metzger², A. Meyer⁵. ¹University of Strasbourg, Service de Physiologie et d'explorations fonctionnelles, Nouvel Hôpital Civil, Centre de Référence des Maladies Autoimmunes Rares, Fédération de Médecine Translationnelle, UR3072, Strasbourg, Strasbourg, France; ²University of Strasbourg, CNRS UMR7104, INSERM U1258, IGBMC, F-67400 Illkirch, Illkirch, France; ³University of Strasbourg, Fédération de Médecine translationnelle, UR3072, Strasbourg, Strasbourg, France; ⁴University of Strasbourg, Service de Physiologie et d'explorations fonctionnelles, Nouvel Hôpital Civil, Fédération de Médecine Translationnelle, UR3072, Strasbourg, Strasbourg, France; ⁵University of Strasbourg, Service de Physiologie et d'explorations fonctionnelles, Nouvel Hôpital Civil, Centre de Référence des Maladies Autoimmunes Rares, Fédération de Médecine Translationnelle, UR3072, Strasbourg, Strasbourg, France

Background: Myositis are rare autoimmune diseases, affecting more women than men, characterized by chronic inflammation of skeletal muscle causing muscle weakness, decreased quality of life and increased mortality.

Glucocorticoids (GC) are potent anti-inflammatory drugs, and are the first line treatment of myositis. They improve muscle strength of myositis patients (therapeutic effect), yet muscle recovery is generally only partial. Moreover, GC have an iatrogenic effect on skeletal muscle fibre leading to steroid myopathy. Thus myositis care has to be improved. Despite the autoimmune terrain of myositis, our team has recently shown that muscle fibres themselves develop immuno-metabolic modifications that participate to muscle weakness and perpetuation of the disease¹. GC effects are mediated by the glucocorticoid receptor (GR), which is expressed in various cell types including immune cells and myofibres, but the cells mediating therapeutic responses remain to be determined.

Objectives: Unravel the mechanisms underlying the therapeutic effect of GC in myositis, particularly elucidate the role of skeletal muscle fibres.

Methods: Experimental myositis was induced in eight to ten week-old C57BL/6J female mice by a single intradermal injection of part of skeletal muscle fast-type C protein along with Freund's adjuvant and an intraperitoneal (IP) injection of pertussis toxin, as previously described². Prednisone (PDN) was administered 14 days (D) after the immunization at 1 mg/kg/day for 7 days by gavage. Mice were euthanized 21 days after myositis induction. Muscle strength was assessed by grip test at D 0, before the 1st PDN administration (D 14) and the day before sacrifice (D 20). To investigate whether the PDN effects are mediated by myofibre, we generated transgenic mice carrying two LoxP sites within the GR gene in muscle, expressing the tamoxifen-inducible Cre-ERT2 recombinase selectively in skeletal muscle fibre (HSA-CreERT2/GR L2/L2). Tamoxifen (1 mg/day for 5 days by IP injection) was administered 9 days after immunization to induce GR ablation selectively in skeletal muscle fibres (GR(i)skm^{-/-} mice). Similar treatments were applied to GR L2/L2 that do not express Cre-ERT2, and served as controls.

We compared 4 groups of myositis mice, GR L2/L2 treated by PDN (n=9) or vehicle (n=9) and GR(i)skm^{-/-} treated by PDN (n=10) or vehicle (n=10), by grip test and at the histological level (hematoxylin-eosin (HE) and Gomori trichrome (GT) staining). Moreover, LC3 expression was studied by RTqPCR and western blot.

Results: Muscle strength was decreased in both GR L2/L2 and GR(i)skm^{-/-} myositis mice from D 14 to D 20. GR L2/L2 myositis mice recovered muscle strength after PDN treatment; no significant difference compared to D 0 was detected. In contrast, PDN did not improve muscle strength in GR(i)skm^{-/-} myositis mice (Figure 1).

HE and GT staining did not reveal quantitative differences in inflammatory infiltrate. Necrotic and degenerative fibres were detected in the 4 groups. At RTqPCR, LC3, an autophagy marker, was upregulated in PDN-treated GR L2/L2 myositis mice compared to untreated GR L2/L2 myositis mice; moreover it was 2-fold more expressed in PDN-treated GR L2/L2 myositis mice compared to PDN-treated GR(i)skm^{-/-} mice.

Conclusion: GR in skeletal muscle fibre is crucial to mediate the therapeutic response to GC in a murine model of myositis. Autophagy is one of the candidate pathways controlled by myofibre GR underlying this effect.

REFERENCES:

- [1] Meyer A et al. IFN- β -induced reactive oxygen species and mitochondrial damage contribute to muscle impairment and inflammation maintenance in

Figure 1. Grip test analysis in GR L2/L2 and GR(i) skm^{-/-} myositis mice. ns, not significant; * $p<0.01$; ** $p<0.001$; *** $p<0.0001$

dermatomyositis. Acta Neuropathol. 2017 Oct;134(4):655-666.

- [2] Sugihara T et al. A new murine model to define the critical pathologic and therapeutic mediators of polymyositis. Arthritis Rheum. 2007 Apr;56(4):1304-14.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2863

OP0248

DEVELOPMENT OF A 3D SKIN MICROTISSUE MODEL FOR FIBROTIC DISEASES

E. Pachera¹, G. Kania¹, A. Juengel^{1,2}, M. Calcagni³, O. Distler^{1,2}. ¹Department of Rheumatology, Center of Experimental Rheumatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland; ²Department of Physical Medicine and Rheumatology, Balgrist University Hospital, University of Zurich, Zurich, Switzerland; ³Department of Hand Surgery, Balgrist University Hospital, University of Zurich, Zurich, Switzerland

Background: Traditional preclinical approaches, such as two-dimensional cell culture and animal models, are often inadequate to mimic the pathophysiological features of complex diseases such as systemic sclerosis (SSc). Human specific targets, such as the recently described pro-fibrotic long non coding RNA (lncRNA) H19X¹, are becoming increasingly relevant in preclinical research, creating the need of new strategies and tools in translational medicine. The employment of novel three-dimensional (3D) culture systems, where multiple cell types are included, is filling an important gap left by the traditional preclinical methods.

Objectives: To develop an easy to produce 3D fibrotic skin microtissues model for translational proof of concept studies.

Methods: Two thousand five hundred dermal fibroblasts isolated from skin of SSc patients were seeded in ultra-low attachment 96-well plates. Fibroblast were let to aggregate into spheres for 48h. Two thousand five hundred primary normal human keratinocytes were added to the culture and let to layer onto the fibroblast spheres for 72h. H19X silencing experiments were used as proof of concept studies. H19X silencing with antisense oligonucleotides or transfections with a scrambled control were performed in fibroblasts prior to the sphere formation for 24h. TGF β (10 ng/ml) was added to microtissue to exacerbate the fibrotic phenotype. Haematoxylin eosin staining as well as immunohistochemistry staining for vimentin and cytokeratin 10 was performed. Skin microtissues were processed for RNA and protein isolation. Pro-collagen I α 1 and fibronectin were quantified in the supernatants with ELISA.

Results: The microtissues presented a core of SSc fibroblast as revealed by vimentin staining and an external layer of keratinocytes as revealed by cytokeratin 10 staining, mimicking the human skin architecture. Gene expression analysis following TGFβ stimulation displayed induced expression of extracellular matrix gene *COL1A1* (p=0.044) and the myofibroblast marker *ACTA2* (p=0.018), indicating that the microtissues were able to develop a fibrotic response. Microtissues, where H19X was silenced, displayed reduced gene expression of *COL1A1* and *ACTA2* after TGFβ stimulation (*COL1A1* p=0.007, *ACTA2* p=0.045). Additionally, H19X silencing led to lower levels of αSMA protein expression (p=0.009) and pro-collagen1α1 secretion (p=0.039) in the supernatant of the microtissue cultures as revealed by Western Blot and ELISA, respectively. *FN1* expression and fibronectin protein levels were not significantly reduced in the microtissues after H19X silencing.

Conclusion: We were able to produce a 3D microtissue resembling skin architecture that can respond to fibrotic stimuli. Knockdown experiments of pro-fibrotic lncRNA H19X confirmed the potential of the model as screening platform for novel pro-fibrotic effectors. A future aim will be to optimize the model for high-throughput automated screening platforms.

REFERENCES:

[1] Pachera, E., et al. (2020). "Long noncoding RNA H19X is a key mediator of TGF-β-driven fibrosis." *The Journal of Clinical Investigation* 130(9): 4888-4905.

Disclosure of Interests: Elena Pachera: None declared, Gabriela Kania: None declared, Astrid Juengel: None declared, Maurizio Calcagni Speakers bureau: Arthrex, Consultant of: Medartis, Arthrex, SilkBiomaterials, Grant/research support from: Medartis, Oliver Distler Speakers bureau: Actelion, Bayer, Boehringer Ingelheim, Medscape, Novartis, Roche, Consultant of: Abbvie, Actelion, Acceleron Pharma, Amgen, AnaMar, Arxx Therapeutics, Bayer, Baecon Discovery, Blade Therapeutics, Boehringer, CSL Behring, ChemomAb, Corpupharma, Curzion Pharmaceuticals, Ergonex, Galapagos NV, GSK, Glenmark Pharmaceuticals, Inventiva, Italfarmaco, iQvia, -Kymera, Medac, Medscape, Mitsubishi Tanabe Pharma, MSD, Roche, Sanofi, UCB, Grant/research support from: Abbvie, Actelion, Bayer, Boehringer Ingelheim, Kymera Therapeutics, Mitsubishi Tanabe
DOI: 10.1136/annrheumdis-2021-eular.3306

OP0249

SERUM PROTEOMIC BIOMARKERS DEFINE PATIENTS WITH SYSTEMIC SCLEROSIS WITH INTERSTITIAL LUNG DISEASE

M. De Santis¹, N. Isailovic¹, A. Ceribelli¹, F. Motta^{1,2}, M. Vecellio^{2,3}, C. Selmi^{1,2}.
¹Humanitas Clinical and Research Center - IRCCS, Rheumatology and Clinical Immunology, Rozzano, Italy; ²Humanitas University, Department of Biomedical Sciences, Pieve Emanuele, Italy; ³University of Oxford, Botnar Research Center, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Oxford, United Kingdom

Background: Systemic sclerosis (SSc) is a systemic condition affecting multiple organs and thus being burdened by high morbidity and mortality; disease management is based largely on the early detection of organ involvement, particularly in the case of interstitial lung disease (ILD), ideally through noninvasive biomarkers. Beside serum autoantibodies associated with diffuse SSc, there is currently no reliable serum marker to predict the onset of SSc organ involvement, monitor its progression, and foresee the response to treatments. Proteomic analysis based on aptamer technology is a powerful method with the potential to address this unmet need in SSc.

Objectives: To identify serum biomarkers associated with ILD in SSc.

Methods: Serum samples from 6 women with SSc (3 with ILD at high-resolution pulmonary CT scan) and 7 age-matched female healthy controls (HC) were analyzed using the SOMAscan platform (SomaLogic, Inc., Boulder, CO, USA) to test more than 1300 proteins even at femtomolar concentration. Subsequent validation of candidate proteins was performed using ELISA in an independent cohort of 88 patients with SSc and 48 HC. Statistical analysis included Student's t-test and was assessed using the SomaSuite software (SomaLogic, Boulder, CO, USA).

Results: The proteomic analysis identified 33 proteins with significantly different serum levels in SSc cases compared to HC and 9 proteins differentiating SSc patients according to ILD (Table 1). Compared to HC, SSc sera manifested an altered expression of proteins involved in extracellular matrix formation and cell-cell adhesion (with higher Calpain, EphA5, IDS, MATN2, MMP-12, TNFR4, and lower desmoglein-1, SNP25), angiogenesis (with higher anti-angiogenic factors as angiopoietin-2 and kininogen high molecular weight) lymphocyte recruitment, activation, and signaling (with higher CXCL-1, LAG3 and lower SH21A) with an overall inhibition of neutrophil function (with lower G-CSF-R, CD177, calgranulin B).

Table 1. Significantly altered proteins at serum proteomic analysis of systemic sclerosis (SSc) with or without interstitial lung disease (ILD) and healthy controls (HC)

SSc versus healthy controls		SSc with ILD versus SSc without ILD and healthy controls	
Increased	Reduced	Increased	Reduced
Aldolase A	Adrenomedullin	FCRL3	BAFF
Angiopoietin-2*	ASGR1	IL-22BP**	DERM
C1QR1	C1s	MCP-3	
Calpain	C5	PDE11	
COLEC12 Eotaxin	Calgranulin B	PGP9.5	
EphA5	CD177	sICAM-5	
Fractalkine/CXCL-1	Desmoglein-1	Stratifin	
Granulins	Flt-3 ligand		
IDS Kininogen, HMV	G-CFS-R		
LAG-3	IL-1Ra		
Lamin-B1	Leptin		
LRP1b	Lypd3		
MATN2	SH21A		
MMP-12	SNP25		
STAT1 TMR4	TPBS2		

*significantly increased also at ELISA** significantly increased at ELISA only in SSc with ILD versus HC

The majority of proteins with higher levels in SSc with ILD compared to SSc without ILD were involved in intracellular signaling and cell cycle (FCRL3, PDE11, Stratifin), along with higher MCP-3, a monocyte chemoattractant, and sICAM-5, ligand for the leukocyte adhesion protein LFA-1. Of note, we found that increased IL-22BP, antagonist of IL-22, and decreased BAFF levels characterized SSc with ILD.

Conclusion: Aptamer proteomic analysis allowed to define serum profiles differentiating SSc patients from healthy controls and SSc with ILD from SSc without ILD; the proteins identified are involved in SSc pathogenic pathways and after further investigation on larger cohorts they can be used as reliable biomarkers.

Characters from table content including title and footnotes: 631

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.3099

Imaging in axial spondyloarthritis – what is new?___

OP0250

MRI VERTEBRAL CORNER INFLAMMATION AND FAT DEPOSITION ARE ASSOCIATED WITH WHOLE SPINE LOW DOSE CT DETECTED SYNDESMOPHYTES: A MULTILEVEL ANALYSIS

R. Stal¹, X. Baraliakos², A. Sepriano¹, F. A. Van Gaalen¹, S. Ramiro¹, R. Van den Berg¹, M. Reijnen³, J. Braun², R. B. M. Landewé⁴, D. Van der Heijde¹.
¹Leiden University Medical Center, Rheumatology, Leiden, Netherlands; ²Rheumazentrum Ruhrgebiet, Rheumatology, Herne, Germany; ³Leiden University Medical Center, Radiology, Leiden, Netherlands; ⁴Amsterdam University Medical Center, Rheumatology, Amsterdam, Netherlands

Background: A few studies have shown an association between vertebral corner inflammation (VCI) and vertebral corner fat deposition (VCFD) on MRI and syndesmophyte formation on cervical and lumbar conventional radiography.

Objectives: To investigate whether magnetic resonance imaging (MRI) patterns of VCI, VCFD and a combination of both are associated with the development of new or grown syndesmophytes as detected by whole spine low dose computed tomography (ldCT), thereby studying these associations also in the thoracic spine.

Methods: Patients in the Sensitive Imaging in Ankylosing Spondylitis cohort underwent MRI at baseline, 1 year and 2 years, and ldCT at baseline and 2 years. MRI lesions were scored by 3 central readers, using the SPARCC method for VCI and the CanDen method for VCFD, and coded as absent or present per timepoint and per reader. MRI patterns over time (Table) were based on patterns studied by Machado et al.¹ and deemed present if seen by ≥2 out of 3 readers. The patterns reflect hypothetical associations between presence and absence of VCI and VCFD, independently and combined, on ldCT detected new or grown syndesmophytes. Individual reader change scores were used for ldCT images, scored by 2 central readers with the Computed Tomography Syndesmophyte Score. New (CTSS 0 to 1, 2 or 3) and grown (CTSS 1 to 2 or 3; 2 to 3) syndesmophytes were grouped together to represent bone formation. Corners not at risk