Abstract OP0090 – Figure 1. Mitochondrial parameters in lung biopsies simultaneously obtained in both, upper and lower lungs, of patients with fibrotic lungs. Lines connect values of the same patients.

Conclusions: Our data support a role of mtDNA-mutations and consecutive respiratory chain dysfunction as a trigger and perpetuator of ROS formation in both, idiopathic interstitial pneumonitis and ILD of patients with systemic sclerosis.

Disclosure of Interest: None declared

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OP0091 CXCL10/CXCL11 SERUM MEASUREMENT AS POTENTIAL PREDICTOR OF SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc), amongst autoimmune rheumatic disorders, shows a heterogeneous and unpredictable course from stable/mild involvement to progressive/late stage, when irreversible multiorgan fibrosis occurs.¹ Early SSc diagnosis remains a clinical challenge; a delay in diagnosis leads, in turn, to therapy delay and more severe patient disability.²⁻³ Earliest vascular immune-mediated alterations are critical in SSc, which, indeed, has been referred to as a 'vascular' disease.⁴ Recognition of biomarker(s) involved in earliest vascular derangements might represent a clinical tool potentially useful for therapeutic approach. Blood level of chemokines IFN γ -inducible protein 10 (IP-10/CXCL10) and IFN-inducible T cell alpha chemoattractant (I-TAC/CXCL11), both involved in endothelial dysfunction, has been shown to associate with worse SSc prognosis.^{5,6}

Objectives: To investigate possible modifications of circulating CXCL10/CXCL11 in the shift from very early diagnosis of SSc (VEDOSS), when vasculopathy and fibrosis are still at very low degree, to definite SSc. Associations between chemokines and capillaroscopic pattern, autoantibody positivity were evaluated.

Methods: Multiplexed luminex technology was used to analyse CXCL10/CXCL11 in total 62 sera, 34 from VEDOSS and 28 from SSc patients, fulfilling the new ACR/EULAR 2013 classification criteria; none of the subjects were treated for SSc. Within VEDOSS group, we selected 29 sera of subjects with follow up (40.67 $\pm$ 5.46 months) and, for each patient of this subcohort, chemokine levels were assessed at follow up (T1) and compared with basal level (T0). Appropriate tests were used for sample distribution and statistical analysis.

Results: Serum CXCL10/CXCL11 were significantly lower in all VEDOSS (CXCL10: 236.00 $\pm$ 40.09 pg/ml; CXCL11: 38.00 $\pm$ 6.97 pg/ml) vs all SSc sera (CXCL10: 633.90 $\pm$ 97.60 pg/ml; CXCL11: 267.70 $\pm$ 76.10 pg/ml; p<0.001 and p<0.01, respectively). Moreover, in VEDOSS subcohort, basal chemokine values (T0) were significantly higher (p<0.001) in sera of subjects who subsequently shifted to SSc (CXCL10: 237.34 $\pm$ 27.34 pg/ml; CXCL11: 45.12 $\pm$ 7.18 pg/ml) vs subjects not developing SSc (CXCL10: 140.06 $\pm$ 16.17 pg/ml; CXCL11: 20.17 $\pm$ 4.06 pg/ml). Sera analysed at follow up (T1) showed a significant increase of both chemokines vs T0 values only in patients who developed SSc (CXCL10: 536.18 $\pm$ 54.98 pg/ml; CXCL11: 250.21 $\pm$ 86.53 pg/ml; p<0.001). CXCL10/CXCL11 retained significant predictive values for SSc development with 165 pg/ml and 29.67 pg/ml cut-off values, respectively, as shown by receiver operating characteristic (ROC) analysis. Significant correlation of CXCL10/CXCL11 with nailfold capillaroscopic pattern was observed.

Chemokine serum level in VEDOSS and SSc patients and predictive chemokine value in VEDOSS subjects. A. Multiplex cytokine assay showed circulating CXCL10 higher in sera of SSc patients vs. VEDOSS subjects (SSc: 633.90 $\pm$ 97.60 pg/ml; VEDOSS: 236.00 $\pm$ 40.09 pg/ml). B. CXCL11 circulating levels were higher in SSc as compared with VEDOSS subjects (SSc: 267.70 $\pm$ 76.10 pg/ml; VEDOSS 38.00 $\pm$ 6.97 pg/ml); *p<0.01; **p<0.001. ROC curves showed that CXCL10 and CXCL11 retained good predictive power to identify VEDOSS subjects later shifting to SSc. C. Serum level of CXCL10 $\geq$ 165 pg/ml was identified as a cut-off value; the area under the ROC curve (AUC) was 0.70 (95% confidence interval-0.52-0.94, p<0.01) with 0.75 sensitivity and 0.70 specificity. D. CXCL11 $\geq$ 29.67 pg/ml was the cut-off value; AUC resulted 0.80 (95% confidence interval-0.67-0.99, p<0.01) with a sensitivity of 0.75 with and a specificity of 0.88.

Correlations	Rho (p)	P
CXCL10/CXCL11	0.6 (Pearson)	<0.0001
CXCL10/NVC	0.6 (Spearman)	<0.0001
CXCL11/NVC	0.5 (Spearman)	<0.001

Serum CXCL10 and CXCL11 concentrations also significantly correlated (Pearson's Rho p=0.6; <0.0001) and correlated with capillaroscopic pattern (Spearman's p 0.6 and 0.5, respectively; p<0.001 and p<0.0001). NVC=nailfold videocapillaroscopy

Conclusions: CXCL10/CXCL11 blood level measurement in VEDOSS patients potentially represents a noninvasive biomarker associated with vascular modifications – as shown by capillaroscopic pattern – predictive of SSc.

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OP0092 DECREASED DICKKOPF-1 EXPRESSION IN CLINICALLY UNINVOLVED SKIN FROM PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: Evidence suggests that the Wnt pathway is a critical mediator of the fibrotic process. The activity of the pathway is tightly regulated by several soluble inhibitors such as Dickkopf-1 (Dkk-1). We, among others, have previously shown that Dkk-1 is absent from scleroderma skin in sharp contrast to skin from healthy subjects where it is clearly expressed.^{1,2}

Objectives: Up until now, Dkk-1 skin expression has only been assessed in established fibrosis, in biopsies obtained from clinically involved areas. We aimed to assess whether the striking lack of Dkk-1 skin expression is also evident in a)

clinically uninvolved skin from patients with SSc and b) very early disease at the puffy/edematous phase, prior to skin thickening.

Methods: Skin biopsies were obtained from a) 12 patients with SSc from lesional skin of the forearm, b) 5 patients with SSc from the upper back which was not affected clinically, c) 2 patients with systemic sclerosis (SSc) with very early disease (<12 months). These patients had puffy hands but no skin thickening; the biopsy was obtained from the distal part of the forearm, in close proximity to the edematous area d) 5 healthy subjects. Dkk-1 expression was immunohistochemically assessed using a mouse anti-human monoclonal antibody (R and D Systems) by a semi-quantitative method (high/moderate/weak/no expression).

Results: Healthy skin displayed a high Dkk-1 immunoreactivity in basal cells of the epidermis as well as in the fibroblasts of the dermis in sharp contrast to clinically involved scleroderma skin that displayed no Dkk-1 immunoreactivity. Clinically uninvolved skin was obtained from 5 patients with SSc (4 diffuse-1 limited) with a median age of 50⁴⁶⁻⁷² and disease duration of 6²⁻¹³ years. In all 5 biopsies Dkk-1 was only moderately expressed in basal cells of the epidermis and dermal fibroblasts. Clinically uninvolved scleroderma skin could be differentiated by immunohistochemical means from both skin from healthy subjects (high Dkk-1 expression) and clinically involved scleroderma skin (no Dkk-1 expression). Skin from very early disease at the edematous phase, prior to skin thickening, displayed only a weak Dkk-1 immunoreactivity in basal cells of the epidermis as well as in the fibroblasts of the dermis.

Conclusions: The decrease in Dkk-1 expression in clinically uninvolved scleroderma skin substantiates previous evidence that the skin in SSc is universally affected underlying the systemic nature of the disease. The downregulation of Dkk-1 at the edematous phase of the disease indicates that the Wnt pathway is involved early in the disease process, prior to establishment of fibrosis a finding with potential pathogenetic implications

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OP0093

LOW RUNX3 EXPRESSION ALTERS DENDRITIC CELL FUNCTION IN PATIENTS WITH SYSTEMIC SCLEROSIS AND CONTRIBUTES TO ENHANCED FIBROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease with unknown pathogenesis manifested by inflammation, vasculopathy, and fibrosis in skin and internal organs. The type I IFN signature found in SSc propelled us to study plasmacytoid dendritic cells (pDCs) in this disease.

Objectives: To identify candidate pathways underlying pDC aberrancies in SSc and to validate its function on pDC biology.

Methods: PCR-based transcription factor profiling and methylation status analyses, SNP genotyping by sequencing, and flow cytometry analysis were performed in pDCs from healthy controls or SSc patients. pDCs were also cultured under hypoxia and RUNX3 levels were determined. To study Runx3 function in DCs, *Itgax-Cre:Runx3^{fl}* mice were used in an *in vitro* functional assays and bleomycin-induced SSc skin inflammation and fibrosis model.

Results: Transcription factor RUNX3 was significantly downregulated in SSc pDCs on RNA and protein levels. A higher methylation status of the *RUNX3* gene correlated with RUNX3 gene expression level and disease susceptibility. After sequencing of the *RUNX3* promoter region, we identified a non-synonymous SNP rs6672420 associated with SSc and hypermethylation of *RUNX3*. Additionally, pDCs cultured in hypoxic conditions showed a significantly lower RUNX3 expression. Furthermore, mouse pDCs deficient of *Runx3* showed enhanced expression of co-stimulatory molecules upon CpG stimulation. Finally, in SSc bleomycin model, mice with DC-specific deletion of *Runx3* showed increased skin inflammation and fibrosis.

Conclusions: We found low RUNX3 expression in pDCs of SSc patients. The presence of a SNP and higher methylation status of RUNX3, and downregulation in hypoxic condition, suggest at least three pathways underlying the low RUNX3 expression observed in SSc pDCs. We demonstrate a detrimental role of RUNX3-abled DCs in a mouse SSc model further underscoring the role of pDCs in this disease. Further research is warranted to explore the potential therapeutic effect of RUNX3 targeting in fibrotic disease.

Disclosure of Interest: None declared

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OP0094

SLIT2/ROBO4 AXIS MAY CONTRIBUTE TO ANGIOGENESIS DISTURBANCE IN SYSTEMIC SCLEROSIS (SSC)

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Background: In SSc, vascular involvement is a primary event characterised by vascular tone dysfunction and microcirculatory abnormalities. Many classes of guidance molecules, such as members of the secreted glycoproteins Slits and their Roundabout (Robo) receptors, play critical roles in angiogenesis. Among these, Robo1 and Robo4 are expressed in endothelial cells. In particular, it has been demonstrated that the interaction of Slit2 with Robo1 promotes angiogenesis, while the Slit2/Robo4 axis inhibits VEGF-mediated endothelial cell migration and tube formation *in vitro* and neovascularization *in vivo*.

Objectives: To evaluate the possible involvement of the Slit/Robo axis in SSc defective angiogenesis.

Methods: Serum Slit2 levels were measured by ELISA in 78 SSc patients, 64 patients with a very early diagnosis of SSc (VEDOSS) and 74 age- and sex-matched healthy controls. Slit2, Robo1 and Robo4 protein expression was evaluated by immunofluorescence in skin biopsies from 15 SSc patients and 10 controls. Slit2 and Robo4 expression in dermal microvascular endothelial cells isolated from 5 SSc patients (SSc-MVECs) and 5 healthy controls (H-MVECs) was analysed by quantitative real-time PCR, Western blot and immunocytochemistry. Proliferation, wound healing and capillary-like tube formation were assessed in H-MVECs challenged with recombinant human (rh) Slit2 or SSc sera (n=6) in the presence/absence of an anti-Slit2 blocking antibody, as well as in SSc-MVECs treated with anti-Slit2 antibody or Robo4 siRNA.

Results: Circulating Slit2 levels were significantly increased in either SSc (median 11.12 ng/ml, IQR 8.02–16.25 ng/ml) or VEDOSS (median 11.27 ng/ml, IQR 8.46–18.60 ng/ml) compared with healthy controls (median 8.79 ng/ml, IQR