of individual parenchymal abnormalities and vascular features measured by QCT methods which associate with mortality in systemic sclerosis (SSc) are currently unknown.

Objectives: To determine whether QCT measures, specifically pulmonary parenchymal abnormalities and pulmonary vascular related structures (PVRS), can predict mortality in SSc and to determine the optimal quantitative thresholds for those parameters.

Methods: A total of 133 subjects (76% women) meeting 2013 ACR/EULAR classification criteria for SSc with a baseline CT within 3 years of diagnosis were retrospectively identified for inclusion. CALIPER (Computer-Aided Lung Informatics for Pathology Evaluation and Rating) was used to quantitatively measure volume of ground glass opacities (GGO), reticular densities, and honeycombing (HC). Total interstitial lung disease (ILD) was the summation of these features. PVRS was also quantified using CALIPER. Values for each feature were expressed as a percentage of total lung volume. Cox models evaluated the hazard ratio (HR) for mortality for each parameter adjusting for age at SSc diagnosis, sex, diffuse SSc subtype, and history of smoking. The optimal thresholds for mortality prediction for each parameter were determined using consensus between 4 methods: Contal and O'Quigley Method, Cox Model Hazard Ratio, Cox Model Wald P-value, and False Discovery Rate. The c-statistic was used to assess each models' ability to predict mortality.

Results: Mean $\pm$ SD for age at SSc diagnosis was 61 ± 13 years and length of follow-up was 4.7 ± 3.0 years. There were 32 deaths (24%). A Cox model including age (HR 1.05, 95% CI: 1.01-1.09), female sex (HR 0.49, 95% CI: 0.22-1.08), diffuse SSc subtype (HR 1.50, 95% CI: 0.69-3.30), and history of smoking (HR 2.09, 95% CI: 0.97-4.53) (Model 1) significantly predicted mortality (C-statistic 0.72, 95% CI: 0.63-0.81). Adjusting for Model 1, reticular densities% (HR 1.19, 95% CI: 1.05-1.35), total ILD% (HR 1.02, 95% CI: 1.00-1.03), and PVRS% (HR 1.19, 95% CI: 1.05-1.35) were associated with mortality on univariable analyses; GGO% (HR 1.01, 95% CI: 0.98-1.04) was not significantly associated with mortality. The optimal thresholds for mortality prediction were then determined and were as follows: GGO $\geq$ 20%, reticular densities $\geq$ 8%, total ILD $\geq$ 20%, and PVRS $\geq$ 5%. While the risk of mortality was significantly increased in subjects with GGO $\geq$ 20% (HR 2.70, 95% CI: 1.21-6.05), reticular densities $\geq$ 8% (HR 4.64, 95% CI: 1.68-12.81), and total ILD $\geq$ 20% (2.59, 95% CI: 1.12-5.99), these baseline thresholds did not improve upon mortality prediction when added individually to Model 1 (C-statistic 0.73 for each). PVRS $\geq$ 5%, which had an over six-fold increase in mortality (HR 6.42, 95% CI: 2.60-15.88), did improve mortality prediction when added to Model 1 (C-statistic 0.78, 95% CI: 0.70-0.86).

Conclusion: PVRS strongly associates with early mortality in patients with SSc and represents a novel radiomic biomarker that provides prognostic information on mortality beyond pulmonary parenchymal abnormalities. CALIPER derived PVRS quantifies CT data through a function that defines connected tubular branching structures. This extracts pulmonary arteries and veins from the adjacent parenchyma but could potentially also include regions of adjoining of fibrosis.¹ Larger studies examining the association between PVRS and progression of cardiopulmonary disease are warranted.

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Systemic sclerosis, myositis - etiology, pathogenesis and animal models

POS0326

ROLE OF THE IL-25 / IL-17RB AXIS IN TH9 POLARIZATION IN PATIENTS WITH PROGRESSIVE SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an inflammatory connective tissue disease leading to chronic and progressive fibrosis, typically affecting the skin and internal organs. The alteration of both innate and adaptive immune responses plays a pivotal role in SSc pathophysiology, although it has not yet been fully elucidated [1].

Recent findings have demonstrated interleukin (IL)-9 overexpression and significant group 2 innate lymphoid cells (ILC2) expansion in patients with SSc. Th9-ILC2-mast cells axis seems to be involved in SSc tissue damage and in the induction of fibrosis [2]. Activation and production of IL-9 by Th9 cells are promoted by transforming growth factor (TGF)- β , thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. Thus, the IL-25 / IL-17RB pathway would act as a key player in SSc.

Objectives: The purpose of this study was to evaluate the role of the IL-25 / IL-17RB axis as a driver in Th9 polarization and ILC2 expansion and polarization in SSc patients.

Methods: 26 patients were enrolled in this study. Peripheral blood and skin biopsy specimens were obtained from SSc patients. PBMCs were isolated and incubated with and without recombinant (r)IL-25 for 24-48-72 hours and the frequencies of Th9 cells, Th17 cells and ILC2 were assessed by flow cytometry analysis. Moreover, the *ex vivo* expression of IL-17RB in ILC2 was also assessed. Immunofluorescence analysis was performed on biopsy skin samples to evaluate IL-17RB expression in ILC2.

Results: In SSc samples, Th9 cells frequency progressively increased after stimulation with rIL-25, compared to healthy controls in which IL-9 frequency decreased over time regardless of rIL-25.

Simultaneously, we evaluated the role of the IL-25 / IL-17RB axis in Th17 cells. In the SSc pool, the initially low rate of IL-17 increased at 72 hours after stimulation with rIL-25. In unstimulated SSc samples, the initially higher IL-17 rate decreased at 72 hours; conversely, it was consistently low in healthy controls, at both baseline and stimulated conditions.

Our results confirmed the presence of IL-25-dependent clonal ILC2 expansion, suggesting a greater and progressive expansion over time in patients with SSc, compared to controls.

Interestingly, increased IL-17RB expression was found in circulating ILC2 from SSc patients supporting the characterization of ILC2 inflammatory phenotype. Consistently, immunofluorescence on the skin of SSc patients showed a marked infiltrate of CD3⁺GATA3⁺ IL-17RB⁺ cells, confirming the presence of the activated inflammatory phenotype ILC2, absent in skin biopsies of healthy controls (Figure 1).

Conclusion: These preliminary data suggest an active role of the IL-25/IL-17RB axis in SSc. It results in Th9 polarization and Th17 clonal expansion, inducing the production of IL-9 and, to a lesser extent, IL-17. Moreover, in addition to promoting Th9-mediated ILC2 differentiation, IL-25 directs the polarization of ILC2 towards the inflammatory phenotype.

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Figure 1. Immunofluorescence on biopsy skin samples of SSc patients (top) and healthy control (bottom).

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POS0327

INACTIVATION OF ALDEHYDE DEHYDROGENASE 3A2 INHIBITS FIBROBLAST ACTIVATION AND TISSUE FIBROSIS

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Background: The aldehyde dehydrogenase (ALDH) superfamily composes a group of 20 enzymes that catalyze aldehyde oxidation. Within this enzyme family, ALDH3A2 stands out for its central role in the oxidation of long-chain aldehydes. Of particular interest, the substrates of ALDH3A2 include also profibrotic lipid mediators such as sphingosine 1-phosphate or leukotrienes, which have been reported to be deregulated in the context of SSc.

Objectives: We aimed to investigate the role of ALDH3A2 in fibrotic tissue remodeling in SSc.

Methods: Fibroblast-to-myofibroblast transition was analyzed by quantification of ACTA2/ α SMA, by assessment of stress fiber formation and mRNA and protein levels of type I collagens. ALDH3A2/Aldh3a2 siRNAs were employed to specifically knockdown ALDH3A2 in dermal fibroblasts both *in vitro* and *in vivo*. Overexpression of ALDH3A2 was achieved by ALDH3A2-pcDNA transfection. The role of ALDH3A2 was investigated in three different mouse models: Bleomycin- and cGvHD-induced dermal fibrosis as well as fibrosis induced by overexpression of a constitutively active TGF β receptor I (TBR1^{CA}). Target genes of ALDH3A2 in fibroblasts were identified by RNA sequencing.

Results: The expression of ALDH3A2 was modestly reduced in dermal fibroblasts of SSc skin as compared to matched healthy controls. This reduction in ALDH3A2 expression was phenocopied by activation of TGF β signaling, whereas selective inhibition of TGF β signaling prevented the downregulation of ALDH3A2 in experimental fibrosis. ALDH3A2 overexpression promoted fibroblast-to-myofibroblast transition with increased levels of α SMA, enhanced formation of stress fibers and reduced collagen release. In contrast, knockdown of ALDH3A2 in dermal fibroblasts inhibited fibroblast activation and collagen release. Moreover, *in vivo* knockdown of ALDH3A2 in the skin of mice ameliorated dermal thickening, myofibroblast differentiation and collagen deposition in three different murine models of skin fibrosis: Bleomycin-induced skin fibrosis and scleroderma GvHD-as models of inflammatory stages of SSc and TBR1^{CA}-induced fibrosis as an inflammation-independent model of SSc. RNA sequencing of ALDH3A2-knockdown fibroblasts demonstrated that ALDH3A2 regulates the activity of a network of profibrotic developmental pathways including TGF β , Wnt, Notch, and Hedgehog signaling.

Conclusion: We demonstrate that ALDH3A2 regulates a network of profibrotic pathways to control fibroblast activation and tissue fibrosis. ALDH3A2 is modestly downregulated in SSc fibroblasts as result of an endogenous, TGF β -driven feedback loop. Although this modest downregulation is not sufficient to counterbalance the aberrant fibroblast activation in SSc, augmentation of this endogenous regulation by knockdown of ALDH3A2 demonstrates potent antifibrotic potential in experimental dermal fibrosis, thereby providing first evidence for ALDH3A2 as a target for antifibrotic therapies.

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POS0328

ENGRAILED 1 COORDINATES CYTOSKELETAL ORGANIZATION TO PROMOTE MYOFIBROBLAST DIFFERENTIATION AND FIBROTIC TISSUE REMODELING

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Background: Engrailed 1 (EN1) is a homeodomain-containing transcription factor with essential roles in embryonic development. In most cell types, the expression of EN1 is restricted to embryonic development. However, under pathological conditions, EN1 can be re-expressed to promote phenotypical adaptation. EN1 is transiently expressed in the developing dermis of murine embryos in a distinct fibroblast lineage and silenced before birth (1). Former EN1-expressing cells give rise to a subpopulation of fibroblasts that has a high capacity for extracellular matrix production in adult murine skin. The role of EN1 in systemic sclerosis (SSc) was previously not explored.

Objectives: To study the role of EN1 in the pathological activation of fibroblasts in tissue fibrosis.

Methods: Bulk RNA-Seq and EN1 or SP1 ChIP-Seq were performed from cultured human dermal fibroblasts. The expression of EN1 was inhibited by siRNA. Cytoskeletal drugs paclitaxel, vinblastin and ROCK inhibitor (Y27632) were used to modulate the cytoskeleton in EN1 knockdown or overexpressing dermal fibroblasts. The role of EN1 in fibroblast activation was evaluated by functional experiments with EN1 knockdown or overexpression in standard 2D culture systems as well as in 3D skin equivalent models. The role of EN1 in skin fibrosis was further studied in En1^{fl/fl} X Col6Cre mice, with fibroblast-specific knockout of En1 in three complementary mouse models: overexpression of a constitutively active TGF β -receptor I (TBR1^{CA}), bleomycin-induced skin fibrosis and TSK1 mice.

Results: Pathologically activated dermal fibroblasts from SSc patients express higher levels of EN1 compared with age and sex matched healthy individuals in the skin and *in vitro*. TGF β induces EN1 expression in fibroblasts in a SMAD3-dependent manner both in cultured fibroblasts and in murine skin. Knockdown of EN1 prevents TGF β -induced fibroblast activation, whereas overexpression of EN1 fosters the pro-fibrotic effects of TGF β with increased expression of α SMA, stress fibers and collagen. RNA sequencing demonstrates that EN1 induces a pro-fibrotic gene expression profile functionally related to cytoskeleton organization and ROCK activation. *In silico* analyses of the promoters of En1 target genes coupled with siRNA-mediated knockdown demonstrated that EN1 regulates these pro-fibrotic target genes by modulating the activity of regulatory modules that contain transcription factors of the specificity protein (SP) family. Functional experiments with selective modulators of ROCK and of microtubule polymerization confirm the coordinating role of EN1 on ROCK activity and the re-organization of cytoskeleton during myofibroblast differentiation in both conventional culture systems and 3D skin equivalents. Consistently, mice with fibroblast-specific knockout of En1 demonstrate impaired fibroblast-to-myofibroblast transition, reduced dermal thickening and impaired collagen deposition in the TBR1^{CA}, bleomycin-induced and TSK1 models.

Conclusion: We characterize the homeodomain transcription factor EN1 as a molecular amplifier of TGF β signaling in myofibroblast differentiation that coordinates cytoskeletal organization in a SP-dependent manner. EN1 might thus be a novel candidate for molecular targeted therapies to interfere with myofibroblast differentiation in fibrotic diseases.

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