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ORAL PRESENTATIONS

Abstracts from the 4th Systemic Sclerosis World Congress
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Session 1 | Pulmonary involvement

CO-01 | ASSOCIATION BETWEEN SKIN GENE EXPRESSION AND CLINICAL PHENOTYPE IN SYSTEMIC SCLEROSIS

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Introduction: Whole-genome DNA microarray analyses of skin biopsies from patients with systemic sclerosis (SSc) have been used to identify four 'intrinsic' subtypes/IS (normal-like, limited, inflammatory and fibroproliferative) based upon gene expression as opposed to the two that are clinically identified and >seven that are identified by analysis of serum autoantibodies. Previously, we showed that SSc patients classified within the inflammatory IS may be most likely patients to demonstrate skin score improvement during mycophenolate mofetil (Cellcept) therapy compared to patients classified in other IS. We hypothesized that IS classification might be better than traditional classification methods for identifying SSc patients with cardiopulmonary disease.
Methods: Gene expression and IS assignment were assessed in 38 SSc patients' biopsies and eleven healthy controls. Detailed clinical assessments were conducted including 2-dimensional Doppler echocardiography with tissue Doppler, high-resolution computed tomography of the thorax (HRCT) and pulmonary function tests within 3mo of clinic visit. Descriptive statistics were determined. Chi-square test of independence or Fisher's exact test was used for categorical covariates, and two-sample t-test or ANOVA was used for continuous covariates. All covariates (adjusted for SSc disease duration [from first non-Raynaud SSc symptom to date of skin biopsy], age, sex, race as appropriate) that were significant at alpha = 0.05 level were considered significant. All statistical analyses were performed in SAS version 9.4 (Cary, NC).
Results: The majority of patients and healthy controls were women (95 and 64%) with diffuse cutaneous SSc (78%). Patients with limited cutaneous SSc were classified within the normal-like (N = 4, 44%), limited (N = 3, 33%) and fibroproliferative (N = 1, 11%) while dcSSc patients were classified within the normal-like (N = 3, 11%), inflammatory (N = 14, 50%) and fibroproliferative (N = 6, 21%) IS. Additionally, four subjects were classified as inflammatory-proliferative because they demonstrated gene expression signatures for both groups. Fibroproliferative subjects had the worst lung disease with the highest HRCT total lung fibrosis score of 11.3 versus 6 (normal-like) and 6.4 (inflammatory), p = 0.03; lowest mean forced vital capacity 69 vs. 93 (normal-like) and 78 (inflammatory), p = 0.0026; and total lung capacity 77 vs. 97 (normal-like) and 91 (inflammatory) % predicted, p = 0.03.
Conclusions: These data suggest that IS classification may indicate points on a continuum of SSc disease with patients moving between inflammatory and proliferative IS. Further they suggest that the proliferation signature is associated with SSc-pulmonary disease. These results have important implications for clinical trial design.

CO-02 | INCIDENCES AND PREDICTORS OF ORGAN MANIFESTATIONS IN THE EARLY COURSE OF SYSTEMIC SCLEROSIS: A LONGITUDINAL EUSTAR STUDY

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Introduction: Systemic sclerosis (SSc) is a rare and clinically heterogeneous autoimmune disorder characterised by fibrosis and microvascular obliteration of the skin and internal organs, particularly the heart, lungs, kidneys and the digestive tract. Organ involvement mostly manifests after a variable period of the onset of Raynaud's phenomenon (RP). Using data from the large, multinational EUSTAR cohort, we aimed to map the incidence and predictors of pulmonary, cardiac, gastrointestinal (GI) and renal involvement in the early course of SSc. renal involvement in the early course of SSc.
Methods: Patients from the EUSTAR cohort with early SSc, defined as those who had a visit within the first year of RP onset were studied. Outcome measures were analysed as a function of time after RP onset using Kaplan-Meier methods, and Cox regression analysis was used to evaluate predictors of incident organ manifestations.
Results: Of the 695 SSc patients in the EUSTAR database who had a baseline visit within one year after RP onset, the incident non-RP manifestations (in order of frequency) were: skin sclerosis (75%), GI symptoms (71%), impaired diffusing capacity for carbon monoxide of <80% predicted (65%), digital ulcers (34%), cardiac involvement (32%), impaired forced vital capacity (FVC) of <80% predicted (31%), increased resting systolic pulmonary artery pressure estimated by echocardiography (PAPsys) >40 mmHg (14%), and renal crisis (3%).
Diffuse skin involvement was a significant predictor of incident FVC impairment, older age and male sex were predictors of incident PAPsys>40 mmHg, and anti-topoisomerase positivity and older age were predictors of incident cardiac manifestations. Incidence rates were highest for diastolic dysfunction, followed by conduction blocks and pericardial effusion. Male sex, anti-RNA-polymerase-III positivity and older age were predictors of renal crisis.
Conclusions: In SSc patients presenting early after RP onset, approximately half of all incident organ manifestations occur within 2 years. These findings may have implications for the design of new diagnostic and therapeutic strategies aimed to 'widen' the still very narrow 'window of opportunity'. They may also enable physicians to counsel and manage patients presenting early in the course of SSc more accurately.

CO-03 | ANTIBODIES AGAINST CHEMOKINE RECEPTORS CXCR3 AND CXCR4 AS MARKER FOR LUNG FIBROSIS IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Introduction: Migration of immune cells plays a pivotal role in inflammation, which is guided by chemokines in sites of inflammation as well as by the presence of their receptors on various immune cells. Chemokine receptors CXCR3 and CXCR4 are involved in fibrotic mechanisms as well as in angiogenesis, which is altered in systemic sclerosis (SSc). It is hypothesized, that IgG antibodies against these receptors are present in patients with systemic sclerosis and are associated with clinical findings.
Methods: 425 sera from 312 consecutive SSc patients and sera from 234 healthy donors were measured for anti-CXCR3 as well as anti-CXCR4 antibodies by ELISA. Ab levels were compared with clinical data from the patients in a cross-sectional as well as longitudinal setting. Protein and mRNA expression of CXCR3 and CXCR4 on monocytes were analyzed by flow cytometry and real-time PCR, respectively, and compared with clinical data.
Results: Anti-CXCR3 and –CXCR4 levels were different among SSc subgroups and healthy donors and were highest in SSc patients with diffuse disease. The levels of the antibodies strongly correlate with each other (r = 0.88). They also negatively correlated with lung function parameters, especially with the percentage values of predicted vital capacity (r = -0.43 and r = -0.5 for anti-CXCR3 and anti-CXCR3 antibodies, respectively, p<0.001). Patients with lung fibrosis and muscular symptoms exhibit higher anti-CXCR3 and anti-CXCR4 ab levels (p<0.05 respectively). Patients with secondary Sjoegren`s syndrome, osteoarthritis, and fibromyalgia exhibited lower anti-CXCR3 antibody levels (p = 0.06, p = 0.05, p = 0.07, respectively). Anti-CXCR4 ab levels were associated with lung fibrosis and muscular symptoms as well as with the presence of cardiac arrhythmias (p = 0.04), systolic pulmonary pressure >25 mmHg as detected by right heart catheter (p = 0.02), and with cardiovascular events (p =0.02). Patients with secondary Sjoegren`s syndrome exhibited lower anti-CXCR4 ab levels. Patients with high anti-CXCR3 and anti-CXCR4 ab levels showed increased odds ratios for further deterioration in their lung function compared to those with low antibody levels. CXCR4 mRNA expression on PBMC was higher in patients with present digital ulcers, with FVC <80%, and in patients with cardiac involvement compared to those without these manifestations.
Conclusions: In conclusion, percentages of PBMC expressing CXCR3 and CXCR4 receptors and the corresponding autoantibodies are linked with clinical manifestations in SSc, mainly with signs of lung fibrosis and with the progress of the disease.

Session 2 | Ulcers

CO-04 | VASCULAR HYPOTHESIS REVISITED: ROLE OF STIMULATING ANTIBODIES AGAINST ANGIOTENSIN AND ENDOTHELIN RECEPTORS IN THE PATHOGENESIS OF SYSTEMIC SCLEROSIS

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Introduction: Systemic sclerosis (SSc) is a severe autoimmune disease linking autoimmunity, vasculopathy, and fibrosis. We have recently induced interstitial lung disease and vasculopathy in C57/Bl6 mice by adoptive transfer of IgG from SSc patients providing proof of concept for a pathogenic role of antibodies in SSc. Here, cross-reactive functional antibodies against the angiotensin II type 1 receptor (AT1R) and the endothelin 1 type A receptor (ETAR) are present in about 85% of the patients fulfilling the old ACR critieria. The antibodies predict mortality, incidental pulmonary arterial hypertension, digital ulcers, and response to therapy suggesting a possible role of the antibodies in SSc-specific disease mechanisms.
Methods: In the present study we analysed factors influencing different effects of the anti-AT1R/ETAR antibodies. ELISAs for the detection of antibody and cytokine levels, wound scratch assays, migration assays, chemotactic assays, cultures of PBMC in the presence of different SSc-IgG fractions, and flow cytometry to measure receptor expression of AT1R and ETAR on PBMC as well as their counterparting receptors AT2R and ETBR were performed. SSc patients were characterized according to the guidelines provided by the German network of systemic sclerosis as well as by EUSTAR.
Results: As shown for endothelial cell regeneration, chemotaxis of T lymphocytes, and neutrophil migration the effects of the antibodies are dependent on the levels of anti-AT1R and anti-ETAR antibodies and were highest in IgG preparations with high anti-AT1R/ETAR antibody levels. IL-8 expression in PBMC was diminished by AT1R and ETAR receptor blockade and was highest by using IgG from SSc patients with early disease compared to those from long-term disease. Expression of AT1R and ETAR in monocytes and lymphocytes from SSc patients was highest in cells from patients with early disease compared to those with late disease. In addition, patients with lung fibrosis exhibited higher mean fluorescence intensity in the monocytic ETBR expression compared to other SSc patients. Finally, concentrations of the profibrotic chemokine CCL18 in the supernatants of PBMC correlate positively with the ratios of AT1R+ and AT2R+ monocytes (r = 0.9) and negatively with the ratios of ETAR+ and ETBR+ monocytes.
Conclusions: In conclusion, the effects of the anti-AT1R and ETAR antibodies are dependent on the antibody levels, clinical features of their donors as well as on receptor expressions of their target cells, which were also dependent on clinical features of the SSc patients. The antibodies offer a novel perspective to investigate mechanisms of the disease.

CO-05 | SENSITIVITY TO CHANGE OF NAILFOLD VIDEOCAPILLAROSCOPY AND RELATIONSHIP WITH DISEASE PROGRESSION

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Introduction: Nailfold videocapillaroscopy (NVC) is a simple, non-invasive and inexpensive imaging technique that allows a detailed assessment of skin microcirculation. Although NVC has face and content validity for the detection of microvascular damages related to systemic sclerosis (SSc), its sensitivity to change has not been assessed in detail. Our aim was to determine in a prospective cohort the merit of NVC to detect meaningful changes over time and whether these changes are associated with disease progression.
Methods: A prospective cohort of 140 SSc patients was recruited over a 12-month period and was followed up on an annual basis for 3 years. NVC was performed at inclusion and repeated once a year by four investigators (ET, GL, CH and AV) according to a standardized procedure. NVC pictures were analysed by one investigator (JA) and classified as early, active and late patterns. Organ progression was defined according to validated definitions. The worsening of the Medsger severity scale (from category 1-2 to category 3-4) was considered as a marker of disease progression.
Results: 140 SSc patients were included (111 women, 56 ± 11 year-old, disease duration of 9 ± 8 years). A change of NVC pattern was observed in 29 SSc patients (21%) during the follow-up period. A progression from a normal or early NVC pattern to an active NVC pattern was detected in 15 patients (10%). Patients who progressed to the active NVC pattern were significantly less at risk to develop ischemic digital ulcers (DU) during follow-up (hazard ratio, HR: 0.78, 95% Confidence Interval, CI 0.17-0.95).
A progression to a late NVC pattern was observed in 14 patients (10%). Progression to a late NVC pattern was associated with the occurrence of new ischemic DU (HR: 4.51, 95% CI 1.68-12.14), lung vascular progression (HR: 5.12 95% CI 1.23-21.27), progression of skin fibrosis (HR: 3.70, 95% CI 1.14-11.94) and the worsening of Medsger disease severity scale (HR: 4.47, 95% CI 1.63-12.26).
Conclusions: Change of the NVC pattern was observed in 20% of patient with SSc during a follow-up of 3 years. NVC has the ability to detect meaningful changes over time associated with markers of disease progression. Our results support the use of NVC for the routine follow-up of SSc patients in order to improve their risk stratification. NVC might be used in the future to select high-risk patients and change to a late NVC pattern might be regarded as potential surrogate marker for disease severity.

CO-06 | DIGITAL ULCERS IN SYSTEMIC SCLEROSIS: PREDICTOR FACTORS

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Introduction: Raynaud's phenomenon and digital ulcers (DU) are frequent among systemic sclerosis (SSc) patients with microangiopathy 40-50% of all patients with SSc.
Our aim was to investigate the endothelial dysfunction assessment (flow-mediated dilatation (FMD), serum levels of Endothelin-1 (ET-1) and ADMA), angiogenesis biomarkers (vascular endothelial growth factor (VEGF), endoglin and endostatin) and microvascular damage in capillaroscopy (NVC) and determine their diagnostic and predictive value for DU in SSc patients.
Methods: Seventy seven SSc patients, divided in two groups: (i) naïve DU patients (39) and (ii) active DU at baseline (38 patients) were followed In a observational cohort study of for 3-years.
Results: Late scleroderma pattern in NVC (AUC:0.846 95% CI: 0.760-0.932), decresed response to shear stress evidenced by low FMD values (AUC:0.754 95% CI: 0.643-0.864), increased serum levels of ET-1 (AUC:0.758 95% CI: 0.649-0.866), ADMA (AUC:0.634 95% CI: 0.511-0.757) and endoglin as well as low angiogenic VEGF serum levels (AUC:0.705 95% CI: 0.579-0.830 were significantly associated to new DU events in the 3-year follow-up.
Cox regression analysis showed that FMD >9.41% (HR:0.37 95% CI: 0.14-0.99); ET-1 >11.85 pmol/L (HR:3.81 95% CI: 1.41-10.26) and late NVC pattern (HR:2.29 95% CI 0.97-5.38) were independent predictors of DU recurrence.
When estimating the probability of occurrence of first DU in naïve DU patients only late scleroderma NVC pattern (HR:12.66 95% CI: 2.06-77.89) was found as an independent predictor factor.
Conclusions: In conclusion late scleroderma pattern in NVC id the best independent risk predictors for new DU. Endothelial dysfunction assessed by FMD and ET-1 were found to be independent predictors of DU recurrence in the 3-year follow-up.

CO-07 | PREVENTION OF DIGITAL ULCERS IN SYSTEMIC SCLEROSIS: REAL LIFE DATA FROM THE DESSCIPHER OBSERVATIONAL STUDY OF THE EUSTAR GROUP

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Introduction: Digital ulcers (DU) are a heavy clinical burden in systemic sclerosis (SSc), therefore their prevention is highly warranted. In the real life clinical practice, the management of DU includes the use of Bosentan or Sildenafil or both, iloprost, as well as other agents including CCB and ACE inhibitors, without strong evidence for their efficacy.
One of the aims of the EC-funded FP7 research project DeSScipher (acronym for "to decipher the optimal management of SSc") was to determine the relative effectiveness of Sildenafil, Bosentan, Sildenafil+Bosentan, Iloprost and calcium channel blockers (CCB)/ACE inhibitors (ACEi) for the prevention of new DU distal to proximal interphalangeal joints in SSc patients.
Methods: Longitudinal data were collected by 28 participating centres in Europe for 24 months. SSc patients with history of DU were analysed to assess the efficacy in secondary prevention of the different treatment arms. For all patients, clinical features, ulcer type and concomitant medications were recorded. Following end-points were considered: time without new DU, proportion of patients without DU, risk of developing new DU and the mean number of new DU for patient at 6 months follow-up.
Results: 1394 patients were enrolled in OT1. 473 out of 1394 patients had history of DU and 268 of them had available follow up data (58.2% limited and 41.8% diffuse SSc subset). 47 out of 268 (17.5%) patients were on Bosentan, 33 (12.3%) on Sildenafil, 40 (14.9%) on Iloprost, 31 (11.6%) on Sildenafil and Bosentan combination and 117 (43.7%) on CCB/ACEi alone. The history of DU was a significant risk factor for developing new DU (OR = 3.146; (95% CI: 1.192-8.306), p = 0.021). In patients with history of DU in the last 24 weeks, the treatment with CCB/ACEi when given alone was associated with 7.1 fold increased risk of developing new DU compared to all other treatment arms (95% CI:1.248-42.85, p = 0.027). Patients on concomitant immunosuppressive treatment had a trend towards worse outcomes. The subanalysis at different time points is still ongoing.
Conclusions: History of DU in the past 24 weeks should trigger an active prevention strategy. In this group, treatment with CCB/ACEi alone is associated with 7 fold increased risk of developing new DU compared to all other treatment arms.

▶ Lecture 1

CO-08 | MACITENTAN RESPONSIVENESS SUPPORTS THE VALIDITY OF A MURINE MODEL OF PULMONARY HYPERTENSION IN SCLERODERMA ASSOCIATED WITH ALTERED TGFBETA/BMPRII SIGNALLING

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Introduction: Pulmonary arterial hypertension (PAH) is an important complication of systemic sclerosis (SSc) that occurs in around 10% of cases. We have previously shown that an imbalance between TGFβ and BMPRII signalling in the transgenic mouse strain TβRIIδk-fib contributes to the development of PH following pulmonary endothelial injury. In this study, we have both prevented and treated PH in this mouse strain using macitentan, an endothelin receptor antagonist licensed to treat PAH in connective tissue disease.
Methods: SU5416 was administered to all TβRIIδk-fib transgenic (TG) mice and littermate wildtype (WT) animals to induce endothelial injury with subsequent endoluminal proliferation and PH in transgenic mice only. Mice were treated daily with either macitentan or vehicle alone (n = 8 each group) from either 2 days before or 8 days following SU5416 administration to assess prevention or treatment respectively. The development of PH in each group was assessed by histology and immunohistochemistry of vessel architecture, *in vivo* haemodynamic studies and RV mass index measurements following 3 weeks of treatment. Microarray analysis of right ventricular tissue was performed with confirmatory qPCR validation.
Results: All TG mice developed a perivascular inflammatory infiltrate and smooth muscle layer hypertrophy after SU5416 administration and pulmonary arteriolar occlusion occurred in 21% of vessels.
RV mass index was elevated in TG animals receiving vehicle compared to other groups. In particular co-administration of macitentan to TG animals treated with SU5416 resulted in normal RV mass (TG vehicle 0.29 ± 0.007, TG macitentan at day -2 0.24 ± 0.007, p<0.05). The increase in RV systolic pressure in TG animals treated with SU5416 was abrogated by macitentan (TG vehicle 28.8 ± 3.2, TG+macitentan at day -2 22.0 ± 2.9, TG+macitentan at day +8, 24.4 ± 1.8, p<0.05) without any change in systemic arterial blood pressure. On histology, no arterial occlusion was seen in TG mice treated with macitentan. Gene expression analysis of whole right ventricle showed alterations in key genes known to be associated with cardiac muscle remodelling and failure. The cluster analysis of TG mice treated with SU5416 compared with those also treated with macitentan is presented.
Conclusions: Macitentan prevents and treats the development of histological and haemodynamic PH in this mouse model of SSc. The pivotal role for perturbed endothelin activity in a model that replicates the imbalance in TGFβ and BMPRII signalling seen in PAH-SSc lung is shown. It underpins the value of this model as a platform for experimental therapeutic studies.

Parallel session 3 | Common clinical situations with poor evidence based data: how to manage?

CO-09 | IMPRESS-2 (INTERNATIONAL MULTICENTRIC PROSPECTIVE STUDY ON PREGNANCY IN SYSTEMIC SCLEROSIS). PROSPECTIVE, CASE-CONTROL STUDY OF PREGNANCY IN SYSTEMIC SCLEROSIS

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Introduction: Data on pregnancy in Systemic Sclerosis (SSc) are limited; we recently published IMPRESS, a retrospective study that compared 109 pregnancies of 99 SSc women with general obstetric population (GOP). In SSc women preterm deliveries (PD, <37W:25% vs. 12%) and severe PD (SPD, <34W:10% vs. 5%), intrauterine growth restriction (IUGR, 6% vs. 1%) and very-low-birth-weight babies (VLBW, 5% vs. 1%) were significantly more frequent than GOP. Multivariable analysis found that corticosteroid use was associated with PD, while folic acid and anti-Scl70+ was protective. The disease remained stable in most SSc patients, but there were four cases of progression within one year from delivery.

Methods: This is a fully prospective, case-control study of 3 cohorts, enrolled at international level: 1) 100 pregnant SSc patients, 2) 200 not-pregnant SSc women (matched for age, auto-antibody pattern, limited/diffuse/sine scleroderma – L/D/S – form and obstetric history), 3) 200 healthy pregnant women (matched for age and obstetric history).We will prospectively investigate disease activity during pregnancy and the further year, pregnancy outcome and children health status at birth and at one year.

Results: 29 pregnancies worldwide are currently ongoing, other 25 patients have already delivered: 9 ACA+(6 L, 1 D, 2 S), 13 Scl70+(7 L, 4 D, 2 S), 1 RNA-P3+D, 2 L without SSc-specific autoantibodies. We observed 4 miscarriages (1 ACA+, 2 Scl70+ and 1 RNA-P3+ patient), 1 therapeutic abortion at W22 (ACA+D in treatment with prednisone 15mg/d and cyclophosphamide), 1 early voluntary termination, 2 SPD in Scl70+L patients (1 cesarean section at W30, due to IUGR and fetal heart rate alteration; 1 spontaneous in twin pregnancy: one twin with VLBW and both with respiratory distress at birth), 2 PD (both SCl70+L at W36), 1 pre-eclampsia at W37 in Scl70+L patient, who developed pulmonary embolism during postpartum.

16 mothers and 28 SSc controls had completed one year surveillance after delivery.We observed disease progression in two cases: the ACA+D patient treated with prednisone and cyclophosphamide showed a kidney function worsening till hemodyalisis, and the Scl70+L patient who underwent twin pregnancy showed significant worsening in pulmonary function testing without specific findings at chest CT.

One child, born at W36 from Scl70+L mother, died at three months due to cardiac malformations and aesopaghus atresia; all other children showed a regular growth at 12 months pediatric evaluation.

Conclusions: IMPRESS-2 is still going on, and we hope to answer important questions: 1) are SSc complications more frequent during pregnancy? 2) Are some autoantibodies protective for prematurity? 3) Which is the prematurity impact on children development? These data will be extremely important for counseling SSc women contemplating pregnancy.

Parallel session 4 | Advances in epigenetics and genetics

CO-10 | INHIBITION OF PHOSPHODIESTERASE 4 (PDE4) REDUCES DERMAL FIBROSIS BY INTERFERING WITH THE RELEASE OF PRO-FIBROTIC CYTOKINES FROM M2-MACROPHAGES

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Introduction: PDE4 catalyses the breakdown of the second messengers cAMP and cGMP to modulate intracellular effects. PDE4 is mainly expressed in inflammatory cells, and its inhibition leads to reduced inflammatory cell activity. In this study, we examined the role of PDE4 inhibition in skin fibrosis and evaluated its potential as an anti-fibrotic target in systemic sclerosis (SSc).

Methods: We studied the anti-fibrotic effects of the PDE4-specific inhibitor rolipram in the models of bleomycin-induced skin fibrosis and sclerodermatous chronic graft versus host disease (sclGvHD), reflecting local and systemic inflammatory fibrotic disease. To better understand the anti-fibrotic activity of PDE4 blockade, we treated fibroblasts and macrophages obtained from healthy individuals and patients suffering from diffuse cutaneous SSc with rolipram and investigated its effects on fibrosis relevant cytokines.

Results: PDE4 inhibition had potent dose-dependent anti-fibrotic effects in bleomycin-induced skin fibrosis and sclGvHD. In bleomycin-induced skin fibrosis, the treatment with the higher dosis (5 mg/kg once daily) of rolipram reduced skin thickness by 36% ($p<0.001$), hydroxyproline content by 42% ($p = 0.004$) and the number of alpha-SMA-positive myofibroblasts by 24% ($p<0.001$). The anti-fibrotic activity of PDE4 inhibition was also prominent in sclGvHD in which we observed reductions of skin thickness by 95% ($p = 0.002$) and of activated myofibroblasts by 24% ($p = 0.002$). The hydroxyproline content showed an almost significant decrease upon PDE4 inhibition. In line with the mode of action of PDE4 blockade, we observed reduced leukocytes counts in bleomycin-induced skin fibrosis (by 37%; $p = 0,012$) and in sclGvHD (by 78%; $p = 0.005$).

Consistent with our *in vivo* findings and the fact that PDE4 is mainly expressed in inflammatory cells, we showed that fibroblasts were not the direct targets of the anti-fibrotic effects of PDE4 blockage. By contrast, PDE4 inhibition decreased the release of pro-fibrotic cytokines IL-6 and 13, TGF-beta1 and beta2, and fibronectin-1 from activated M2 macrophages obtained from healthy volunteers and SSc patients, resulting in reduced fibroblast activation and collagen release.

Conclusions: PDE4 inhibition reduces the release of pro-fibrotic cytokines from M2 macrophages, which leads to decreased fibroblast activation and collagen release. Importantly, rolipram is a lead compound of the PDE4 inhibitor apremilast, which is approved for the treatment of psoriatic arthritis. Therefore, our preclinical findings might prompt the use of PDE4 inhibitors in clinical studies with patients suffering from SSc, in particular those with inflammation-driven fibrosis.

CO-11 | EPITHELIAL FLI1 DELETION INDUCES FIBROSIS AND AUTOIMMUNITY WITH DOWNREGULATION OF AIRE – POSSIBLE ROLES IN SYSTEMIC SCLEROSIS PATHOGENESIS

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Introduction: Since systemic sclerosis (SSc) is characterized by the three cardinal pathological features, such as fibrosis, vasculopathy, and autoimmunity, the studies on this disease have focused on fibroblasts, endothelial cells, and immune cells. However, recent studies have demonstrated that SSc keratinocytes have a disease-specific phenotype contributing to the development of skin fibrosis. So far, a series of studies from our laboratory demonstrated the critical role of transcription factor Fli1 in the pathogenesis of SSc, especially in the induction of SSc-like phenotypes in fibroblasts, vascular endothelial cells, and macrophages. During these studies, our preliminary

data showed a marked downregulation of Fli1 in keratinocytes of SSc lesional skin. Based on these backgrounds, to investigate the significance of Fli1 deficiency in keratinocytes in the pathogenesis of SSc, we generated keratin 14 (K14) conditional Fli1 knockout mice and investigated if these mice develop SSc-like phenotypes.
Methods: Keratinocyte-specific Fli1 knockout mice were generated by crossing Fli1flox/flox mice with mice expressing Cre recombinase under the control of K14 gene promoter. The phenotype of these mice was investigated by histologic examination, immunohistochemistry, immunofluorescence, hydroxyproline assay, enzyme-linked immunosorbent assay, quantitative reverse transcription PCR, and flow cytometry. *In vitro* assays, including immunoblotting, quantitative reverse transcription PCR, chRomatin immunoprecipitation, DNA affinity precipitation, and reporter assay, were also carried out with cultivated keratinocytes.
Results: These mice histologically exhibited not only enhanced skin fibrosis, but also esophageal fibrosis and pulmonary fibrotic changes with inducible bronchus-associated lymphoid tissue. Furthermore, serum immunoglobulin levels were remarkably elevated and anti-nuclear antibodies, including anti-topoisomerase I antibody, were detectable. Consistent with these strong autoimmune signatures, the analysis of thymi, where K14 is also abundantly expressed in their medullary epithelial cells, demonstrated a significant decrease in Autoimmune regulator (AIRE) gene expression in these mice. Importantly, Fli1 served as a potent activator of the AIRE gene.
Conclusions: These results indicate the pathological significance of Fli1 deficiency in the epithelial cells in SSc. We herein report our data and discuss its pivotal contribution to the pathogenesis of SSc.

CO-12 | ENDOTHELIAL-TO-MESENCHYMAL TRANSITION (ENDOMT) AS A KEY PROFIBROTIC SWITCH IN SYSTEMIC SCLEROSIS (SSC)

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Introduction: Profibrotic myofibroblasts may arise from different sources, including expansion and activation of resident tissue fibroblasts, recruitment of bone marrow-derived circulating precursors and transition of epithelial cells into mesenchymal cells. Endothelial cells (ECs) may also exhibit substantial plasticity by undergoing EndoMT, a transdifferentiation by which ECs disaggregate, lose polarity and acquire myofibroblastic features. EndoMT is a phenotypic conversion in which ECs lose their specific markers such as CD31, von Willebrand factor and VE-cadherin, and acquire mesenchymal cell products such as α-smooth muscle actin (α-SMA), S100A4/fibroblast-specific protein-1 and type I collagen. EndoMT can be induced *in vitro* by transforming growth factor (TGF)-β and is involved in experimental skin fibrosis. In the present study, we investigated whether EndoMT may actively participate in the pathogenesis of dermal fibrosis in SSc.
Methods: Dermal microvascular ECs (dMVECs) were obtained from skin biopsies from 6 patients with diffuse cutaneous SSc (dcSSc) and from 6 healthy subjects (SSc-dMVECs and H-dMVECs, respectively). CD31-positive cells were subjected to immunomagnetic isolation. H-dMVECs were left untreated or treated for 48 and 72 hours with recombinant human TGF-β or sera from dcSSc patients (n = 6) and healthy subjects (n = 6). The expression of CD31, VE-cadherin, α-SMA, S100A4, type I collagen and Fli1 and Snail1 transcription factors was evaluated by quantitative real-time PCR, Western blotting and immunofluorescence staining. Skin sections from SSc patients (n = 12) and healthy subjects (n = 10) were subjected to CD31/α-SMA and VE-cadherin/α-SMA double immunofluorescence staining.
Results: H-dMVECs exhibited a typical EC morphology with a large and flattened shape, whereas the majority of SSc-dMVECs had an elongated shape often characterized by many branches. Both H-dMVECs and SSc-dMVECs were homogeneously positive for the pan-EC marker CD31. However, the expression of CD31, VE-cadherin and Fli1 was markedly decreased in SSc-dMVECs compared with H-dMVECs. SSc-dMVECs co-expressed α-SMA, S100A4 and type I collagen, which were instead almost undetectable in H-dMVECs. Double immunofluorescence staining clearly revealed the presence of numerous CD31-positive cells displaying α-SMA stress fibers in SSc-dMVEC cultures. Strong expression and nuclear localization of Snail1 were constitutively detected in SSc-dMVECs. Treatment of H-dMVECs with TGF-β or dcSSc sera markedly inhibited the expression of CD31, VE-cadherin and Fli1 in parallel with the induction of α-SMA, S100A4, type I collagen and Snail1. Histologic assessment of skin sections identified the exclusive presence of CD31/α-SMA and VE-cadherin/α-SMA double-positive ECs in SSc dermal vessels.
Conclusions: These findings provide the first direct evidence that EndoMT may contribute to the development of dermal fibrosis in SSc.

CO-13 | WNT/PCP-SIGNALING AS A β-CATENIN-INDEPENDENT PATHWAY TO PROMOTE FIBROBLAST ACTIVATION IN SSC

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Introduction: From our previously studied we has been identified that canonical Wnt/beta-catenin signaling as a core pathway of fibrosis in SSc. However, the non-canonical Wnt signaling pathways have not yet been analyzed. A subset of Wnt proteins can bind to different cell surface receptors and activate non-canonical Wnt pathways which are independent of beta-catenin pathway. In this study we aimed to characterize the role of Wnt5a in fibrotic diseases.
Methods: We used qPCR, IF and Western blot to check the expression level of non-canonical Wnt ligands from patients with SSc, IPF and healthy controls as well as in experimental fibrosis. The effect of fibroblast-specific (col1a2; CreER) as ubiquitous (ubiquitin; CreER) knockout of Wnt5a was evaluated in bleomycin-induced fibrosis and in experimental cGvHD. Wnt5a-induced signaling was analyzed *in vitro* and *in vivo* using small molecule inhibitors, siRNA mediated knockdown, Western blots and reporter assays.
Results: The mRNA and protein levels of Wnt5a, but not the others non-canonical Wnt ligands, are increased in fibrotic skin of patients with SSc and in the lungs of patients with IPF as compared to healthy volunteers. Wnt5a was also upregulated in bleomycin-induced skin and lung fibrosis. Wnt5a is strongly expressed in fibroblasts in fibrotic tissues. Overexpression of Wnt5a induced pulmonary and dermal fibrosis *in vivo* and promoted myofibroblast differentiation and collagen release *in vitro*. Fibroblast-specific knockout of Wnt5a ameliorated bleomycin-induced fibrosis and experimental cGvHD. The pro-fibrotic effects of Wnt5a are mediated by Wnt/PCP signaling via binding with Ror2 and Ryk receptors and subsequent phosphorylated JNK and cJun, but independent of Wnt/beta-catenin and Wnt/Calcium signaling. Consistently, inactivation of JNK or cJun by pharmacologic or genetic methods abrogated the pro-fibrotic effects of Wnt5a *in vitro* and *in vivo*.
Conclusions: These findings characterize Wnt5a as a novel mediator of fibroblast activation in SSc. Upregulation of Wnt5a induces fibroblast activation and promotes tissue fibrosis. The pro-fibrotic effects of Wnt5a are independent of canonical Wnt signaling and are mediated by PCP signaling via binding with Ror2 and Ryk receptor and JNK/cJun activation.

Parallel session 5 | Cardiac

CO-14 | INFLAMMATORY CELLS AND STROMAL SUB-POPULATIONS INVOLVEMENT IN THE MYOCARDIAL FIBROGENESIS IN SYSTEMIC SCLEROSIS

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Introduction: Clinical and post mortem investigations demonstrated myocardial lesions in more than 50% of SSc patients. Cardiac involvement described in SSc patients resembles inflammatory dilated cardiomyopathy (iDCM). Myofibroblasts are the main players in cardiac fibrogenesis, but their origin remains unknown. Here, we aim to determine the role of inflammatory and stRomal cells in myocardial remodeling in SSc.

Methods: Immunohistochemistry (IHC) and immunofluorescence (IF) were performed on paraffin sections or cryosections of hearts from Fra-2 tg mice and controls. Flow cytometry analysis was implemented to individuate and characterize subsets of myocardial inflammatory and stRomal cells. Different stRomal cell subsets were sorted, cultured and stimulated with TGF-b1. Differentiation and proliferation potentials were assessed by qPCR, proliferation assays, IF and stress fibers staining on sorted cells. The antisense oligonucleotide GAPMER was used to downregulate Fra-2 in stRomal cells.
Results: The Fra-2 tg mouse model of SSc/iDCM spontaneously developed myocardial inflammation with CD45+ leukocyte infiltrates and fibrosis with collagen deposition, starting at week 13. Myocardium of SSc/iDCM patients and Fra-2 tg mice showed an increased expression of the transcription factor Fra-2 among the inflammatory and fibrotic regions.
Flow cytometry analysis revealed four myocardial stRomal cell (Ter119-/CD45-/CD31-) populations: gp38+CD90.2-, gp38+CD90.2+, gp38-CD90.2+ and gp38-CD90.2-. The frequency of the populations gp38+CD90.2- (single positive cells) and gp38+CD90.2+ (double positive cells) was significantly higher in Fra-2 myocardium compared to control mice (both p = 0.0091). Of note, gp38+ stRomal cells co-localized with myofibroblasts as indicated by the marker a-smooth muscle actin (aSMA) in Fra-2 heart.
Single and double positive myocardial stRomal cells were successfully propagated *in vitro*. Interestingly, both of them up-regulated mRNA levels of aSMA after 7 days of TGF-b1 treatment. Furthermore, stRomal cells from Fra-2 tg mice showed the presence of aSMA fibers and stress fibers even without TGF-b1 stimulation indicating that they might be activated towards the myofibroblast phenotype. Moreover, we observed increased proliferation capacity of Fra-2 stRomal cells compared to controls.
Besides, the Fra-2 myocardium displayed a 2-fold increase of inflammatory CD45+/CD11b+ monocytes (p = 0.0167), which showed an increased expression of fibronectin.
Fra-2 downregulation by antisense GAPMER transfection in myocardial gp38+ stRomal cells resulted in the significant downregulation of fibrotic genes: aSMA, collagen I and fibronectin confirming its importance during myocardial fibrogenesis.
Conclusions: We suggest that inflammatory and stRomal cells might play a pivotal role in TGF-b1/Fra-2 driven myocardial remodeling. Targeting of identified key-cellular sources of myocardial fibrogenesis in SSc animal models might serve the basis for developing effective therapies.

CO-15 | N-TERMINAL PRO BRAIN NATRIURETIC PEPTIDE IS A STRONG PREDICTOR OF MORTALITY IN SYSTEMIC SCLEROSIS

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Introduction: If the global prognosis of Systemic Sclerosis (SSc) remains poor, it is apparent that some subsets of patients have very bad outcomes while some others remain stable with a milder form of the disease. The accurate prediction of outcomes in this polymorphic disease is therefore a critical unmet need. Cardiovascular involvement is a key contributor to mortality. Therefore, we herein investigated whether the cardiac biomarker NT-proBNP could be used for the prognostic assessment in SSc.
Methods: This is a longitudinal, multicenter study that included 523 patients with SSc (aged 54 ± 13 years, 8 ± 9 years disease duration, diffuse cutaneous form in 168). Plasma NT-proBNP was measured at baseline and SSc patients were prospectively followed up yearly to 5 years. The rates of all-cause mortality at 3 (primary endpoint) and 5 years were determined.
Results: At baseline, 37 patients had major cardiovascular involvement, including 17 with pulmonary arterial hypertension (PAH) and 20 with reduced left ventricular ejection fraction (LVEF <55%). No patient was lost of follow-up and 32 patients (7%) died within 3 years and 59 (16%) within 5 years.
NT-proBNP was increased in patients who died within 3 years versus in those who survived (203 129-514 versus 88 47-167 ng/l; p<0.001). Similar results were observed for the 5-year follow-up period with baseline concentrations in patients who died vs who survived of 148 73-362 versus 85 41-168 ng/l; p<0.001). Using a 125 ng/l cutoff value, NT-proBNP reliably predicted 3-y and 5-y mortality; respective sensitivities of 78.1% and 59.3%, respective negative predictive values of 97.6% and 90.0% respectively). In multivariate analyses, elevated NT-proBNP was an independent predictor of mortality at 3 years (p = 0.03) such as pulmonary functional tests (FVC, p = 0.008 and DLCO/VA, p = 0.01).
In a total of 434 patients, no major cardiovascular complication was observed at baseline. In this sub-population, NT-proBNP concentration was 85 (44-157) ng/L in those who survived at 3 years versus 188 (129-383) ng/L in those who deceased, p<0.001; respective proportions of patients with increased NT-proBNP >125 ng/L were 122 (32.9%) versus 19 (82.6%), p<0.001. In multivariate analysis, elevation of NT-proBNP remained associated with 3-y mortality (p = 0.001; as well as FCV, p = 0.003). Similar results for 5-y mortality were observed.
Conclusions: Our study demonstrates that NT-proBNP is strongly and independently associated with 3-y and 5-y mortality and that 125 ng/L is confirmed as a threshold value.

CO-16 | CARDIAC BIOMARKERS IN PRIMARY HEART INVOLVEMENT IN SYSTEMIC SCLEROSIS: NT-PROBNP AND TROPONIN T AS DIAGNOSTIC AND PROGNOSTIC BIOMARKERS

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Introduction: Cardiac involvement is the leading cause of death in about one third of patients with Systemic Sclerosis (SSc). The aim of our study was to define the diagnostic and prognostic role of cardiac troponin T (cTnT) and NT-proBNP to identify primary cardiac involvement and SSc-patients with higher risk of cardiac death.
Methods: High sensitivity cTnT and NT-proBNP were measured in 245 consecutive SSc-patients and in age-sex-matched healthy controls. All SSc-related deaths were registered during a mean follow-up of 37.0 ± 19.9 months.
Results: cTnT levels and NT-proBNP values were higher in SSc-patients (cTnT: 0.03 ± 0.006 ng/ml and NTproBNP: 898.8 ± 3063.8 pg/ml) than in healthy controls (cTnT: 0.006 ± 0.0004 ng/ml and NT-proBNP: 90.6 ± 70.9 pg/ml, p<0.00001 for both comparisons). Considering 195 SSc-patients without pulmonary arterial hypertension or renal failure, cTnT levels were upper the limit in 63(32.3%), 45 of whom (72.6%) complained of cardiac symptoms, while 17(27.4%) were asymptomatic. NT-proBNP levels were above the cut-off limit of 125 ng/ml, recommended by the manufacturer, in 62 patients (31.8%) and 37(62.7%) presented also increased levels of cTnT. The levels of NT-proBNP directly correlated with the cTnT levels (R = 0.5; p<0.0001).
At univariate analysis, high levels of cTnT were associated with diffuse skin disease, restrictive lung syndrome, left ventricular ejection fraction (LV-EF)<55% on echocardiography, long QTc and right bundle branch block (RBBB), and at multivariate analysis RBBB[RR: 7.0(1.2-41.1)] and diffuse skin disease[RR: 2.0(1.1-4.2)] emerged as independent predictors of high cTnT levels. At univariate analysis increased levels of NT-proBNP were associated with diffuse skin involvement, history of digital ulcers, LV-EF<55% and restrictive lung syndrome, while at multivariate analysis age[RR: 1.1(1.03-1.2)] and diffuse disease[RR: 2.3(1.1-4.9)] emerged as independent predictors of increased NT-proBNP.
During the follow-up, 14 SSc-related death occurred; 9 of these were directly related to cardiac involvement (sudden cardiac death or heart failure). Cumulative survival estimated by Kaplan-Mayer curve was worse in patients with increased baseline levels of cTnT (X2 = 10.2, p = 0.001) and NT-proBNP (X2 = 11.1, p = 0.001). Patients with high levels of both biomarkers, cTnT and NT-proBNP, had the worst survival (X2 = 13.5, p = 0.003). At Cox regression analysis on survival for SSc cardiac involvement elevation of cTnT and NT-proBNP was independently associated with bad outcome [RR: 9.8(1.2-78.6)].
Conclusions: cTnT and NT-proBNP identify patients with subclinical heart disease and allow to define a poor cardiac outcome and to guide further specialistic imaging and therapy.

CO-17 | PROGNOSTIC ROLE OF VENTRICULAR ECTOPIC BEATS IN SYSTEMIC SCLEROSIS

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Introduction: Arrhythmias are frequent in Systemic Sclerosis (SSc) and portend a bad prognosis, accounting alone for 6% of SSc total deaths.
Methods: We performed a prospective cohort study to define the role of 24h-ECG-Holter in the identification of patients at high risk of life-threatening arrhythmias and sudden cardiac death (SCD) in SSc. 100 SSc-patients with symptoms and/or signs suggestive of cardiac involvement underwent 24h-ECG-Holter. The primary end-point was a composite of SCD or the need for implantable cardioverter defibrillator (ICD). A mean follow-up of 23.1 ± 16.0 months was reached.
Results: Fifty-six patients (56%) had 24h-ECG-Holter alterations and 24(24%) presented frequent ventricular ectopic beats (VEBs), classified as polymorphic in 11 (26.2%). The mean number of VEBs was strikingly high (2046.1 ± 6027.8//24h), with a maximum of 33615/24h. Supraventricular ectopic beats (SVEBs)>1000/24h were also frequent (19%), with a mean number of 798.9 ± 1835.6 per day; seventeen patients (17%) presented runs of SVEBs. Fourteen patients (14%) presented episodes of supraventricular paroxysmal tachycardia (SVPT), with a maximum of 36 beats, while 11 patients (11%) exhibited runs of NS-VT, the longest of 34 beats. The number of VEBs correlated with cardiac troponin T (cTnT) levels (R = 0.4, p<0.001) and inversely correlated with left ventricular ejection fraction (LV-EF) on echocardiography (R = -0.4, p<0.001). Furthermore, the number of VEBs directly correlated with severity index and with the extension of skin involvement evaluated by the mRSS (p = 0.3 for both correlations).
During the follow-up, 5 patients died of SCD and two required ICD-implantation for life-threatening arrhythmias. The 7 patients who met the composite end-point had a higher number of VEBs, higher levels of cTnT and NT-proBNP and lower LV-EF (p = 0.001 for all correlations), while in all of them a pulmonary arterial hypertension was ruled out by right heart catheterization. All 7 patients had frequent ventricular ectopy at baseline ECG-Holter, while LV-EF range in patients who met the primary end-point was wide and LV-EF was not reduced in all. At ROC curve, VEBs>1190/24h showed 100% of sensitivity and 83% of specificity to predict the primary end-point (AUROC = 0.92, p<0.0001). Patients with VEBS>1190/24h had lower LV-EF and higher cTnT levels compared to patients with VEBs<1190/24h.
Conclusions: VEBs are frequent in SSc and correlate with cardiac damage; VEBS>1190/24h identify patients at high risk of major arrhythmic complications. 24h-ECG-Holter should be considered as a part of routine evaluation in SSc-patients with suspected cardiac involvement and it could become an additional risk-stratification test to select SSc-patients at high-risk of SCD, in whom an ICD-implantation could represent a potential life-saving intervention.

CO-18 | EARLY DETECTION OF CARDIAC INVOLVEMENT BY MAGNETIC RESONANCE: CLINICAL CORRELATIONS IN SYSTEMIC SCLEROSIS

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Introduction: Cardiac involvement in systemic sclerosis (SSc) affects the prognosis of the disease. Myocardial fibrosis is the pathological hallmark of this complication and has been reported in >50% of cases in necropsy. Echocardiography is the routine imaging tool to easily detect cardiac involvement, but it is not accurate to detect myocardial fibrosis. Delayed gadolinium enhancement (DE) cardiovascular magnetic resonance (CMR) is the gold-standard for myocardial fibrosis assessment.
Our aim was to evaluate the added value of DE-CMR to echocolorDoppler and its clinical correlations in SSc patients.
Methods: After a thorough clinical characterization, 208 SSc patients (age = 51 ± 14, 91% females) underwent on the same day a comprehensive echocardiogram, including tissue Doppler imaging (TDI), and a DE-CMR.
Results: A reduction in ejection fraction was detected in <3% patients, whereas DE-CMR showed a pattern of non-ischaemic myocardial fibrosis in 30% patients. In 5 patients, T2-weighted CMR showed myocardial edema, which resolved after steroid therapy. A correlation was found between DE and ventricular abnormalities at ecg Holter monitoring, with the majority of patients with ecg Holter alterations showing DE (65% vs 35%, p<0.01). Among clinical characteristics, only a history of ulcers (OR 5.66; 95% CI 1.38-23.21) was independent predictors of the presence of myocardial fibrosis.
Conclusions: Subclinical cardiac involvement is frequent in SSc. CMR can detect patterns of reversible (by T2-weighted) and irreversible (by DE) cardiac involvement, often without detectable systolic and diastolic left ventricular dysfunction. The majority of patients with ventricular alterations at ecg Holter monitoring show DE, underlining the potential role of myocardial fibrosis in the genesis of ventricular arrhythmias. There is an association between a history of ulcers and myocardial fibrosis that may suggest a similar pathological substrate of abnormal vascular function, underlying digital and cardiac complications.

CO-19 | IMAGING PATTERNS OF STRESS PERFUSION-FIBROSIS IN SYSTEMIC SCLEROSIS USING CARDIOVASCULAR MAGNETIC RESONANCE

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Introduction: Perfusion's defects and consequent fibrosis are major causes of cardiac disease in scleroderma (SSc). We hypothesized that using stress perfusion-fibrosis cardiovascular magnetic resonance (CMR), we can identify the imaging pattern of heart involvement during various stages of SSc.
Methods: 60 patients (52F/8M) with diffuse SSc were evaluated. Twenty-two out of 60 had a recently diagnosed SSc (RSSc)(<2 yrs), while 38/60 had a long-standing SSc (LSSc) (>5 yrs). ECG breath hold cine and stress perfusion-fibrosis protocol were assessed by 1.5T system. Stress perfusion-fibrosis was performed using 140 mg/kg/min adenosine for 4 minutes and 0.1 mmol/kg Gd-DTPA was given during the first-pass perfusion sequence. A rest perfusion was performed using the same protocol. Late gadolinium enhanced images (LGE) were obtained 15 minutes after intravenous gadolinium-DTPA for fibrosis detection.
Results: A reduction in Myocardial Perfusion Reserve Index (MPRI) was identified in 19/22 RSSc and in 33/38 LSSc, which correlated only with the presence of digital ulcers (p<0.05). MPRI was lower in LSSc compared to both RSSc and controls (0.6 ± 0.2 vs 1.2 ± 0.4 vs 3.9 ± 0.8, respectively). Myocardial fibrosis, identified by LGE, was detected in 5/22 RSSc and 28/38 LSSc and presented linear, nodular, subepicardial and diffuse subendocardial pattern.
Conclusions: We found that reduction in MPRI is more common in patients with RSSc than myocardial fibrosis indentified by LGE which in contrast was predominantly noticed in LSSc group. Whether MPRI is an earlier marker of heart involvement in SSc and the potential clinical implications of this observation remain to be determined in future studies.

Parallel session 6 | Current research in immunity / inflammation

CO-20 | PAN PPAR AGONIST IVA337 IS EFFECTIVE IN PREVENTION AND TREATMENT OF EXPERIMENTAL SKIN FIBROSIS

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Introduction: The pathogenesis of systemic sclerosis (SSc) involves a distinctive triad of autoimmune, vascular and inflammatory alterations resulting in fibro-

sis. Evidence suggest that peroxisome proliferator-activated receptors (PPARs) play an important role in SSc-related fibrosis and their agonists may become effective therapeutic approaches. The objective of this study was to determine the expression of PPARs in human fibrotic skin and investigate the effects of IVA337, a pan PPAR agonist, in *in vitro* and *in vivo* models of fibrosis.
Methods: The anti-fibrotic effects of IVA337 were studied using a bleomycin-induced mouse model of dermal fibrosis. The *in vivo* effect of IVA337 on wound closure and inflammation were studied using an excisional model of wound healing.
Results: Lower levels of PPARα and γ were detected in the skin of SSc patients compared to controls. In mice, IVA337 treatment was associated with decreased extracellular matrix (ECM) deposition and reduced expression of phosphorylated-SMAD2/3 – intracellular effector of TGF-β1. Although the anti-fibrotic effect of pan PPAR was similar to that of PPAR-γ agonist alone, a significant downregulation of several markers of inflammation was associated with IVA337. Despite its anti-inflammatory and anti-fibrotic properties IVA337 did not interfere with wound closure. *In vitro* effects of IVA337 included inhibition of cellular migration, attenuation of transcription of ECM genes and alteration of TGF-β signaling pathway through decreased p-SMAD2/3.
Conclusions: These findings indicate that simultaneous activation of all three PPAR isoforms exerts a dampening effect on inflammation and fibrosis making IVA337 a potentially effective therapeutic candidate for the treatment of fibrotic diseases including SSc.

CO-21 | MULTI-ORGAN SYSTEMS BIOLOGY ANALYSIS OF SYSTEMIC SCLEROSIS REVEALS A MACROPHAGE SIGNATURE ASSOCIATED WITH DISEASE SEVERITY IN MULTIPLE END-TARGET TISSUES

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Introduction: Systemic sclerosis (SSc) is characterized by multi-organ involvement and clinical heterogeneity. "Big data" approaches have yielded powerful tools to infer tissue-specific pathobiology. Large amounts of SSc gene expression data have been generated from different tissues and sample patients with distinct SSc clinical pathology. We performed an integrative meta-analysis of ten different SSc gene expression datasets that identifies common disease drivers and infers the sequence of pathological events using samples from patients with early and late disease. We present a network model of SSc-associated pulmonary fibrosis (SSc-PF) progression that has parallels with the skin network suggesting commonalities of the pathological processes.
Methods: We developed and employed a novel data mining procedure that identified conserved coexpression patterns between ten datasets from four different tissues (skin, lung, esophagus, blood) with multiple clinical manifestations (pulmonary arterial hypertension [PAH], PF, limited and diffuse subtypes). We identified genes and processes that were conserved across all solid tissues and were highly expressed in pulmonary manifestations of SSc. We used these modules to query tissue-specific gene-gene interaction networks and analyzed the resulting lung- and skin-specific networks to infer common and tissue-specific fibrotic and inflammatory pathways.
Results: We identified a common gene signature indicative of macrophages that contribute to the extracellular matrix (ECM) remodeling processes. This signature was found in PAH and PF in lung, as well as the inflammatory molecular subsets in skin and esophagus. Analysis of the tissue-specific networks revealed a coupling of inflammatory and ECM processes in solid tissues that was absent in PBMC samples. Projection of genes that were highly expressed in early and late SSc-PF onto the lung network showed a lung macrophage lipid trafficking signature that was up in early, but not late disease. We also found evidence for a shift in the balance of apoptotic processes in late disease.
Conclusions: We find evidence in our analysis and present a model of SSc-PF for an initial insult that results in an interferon response, followed by lipid uptake by lung macrophages that are alternatively activated and may secrete TGFbeta. The lung network show strong similarities in the overall process, but also distinctions, which suggests this model may reflect the process of fibrosis in all SSc end-target tissues. Genes that bridge multiple molecular processes (e.g. ECM remodeling, apoptosis) have been tested experimentally and shown to be important in animal models of PF and are attractive targets for therapeutic intervention.

CO-22 | INNATE LYMPHOID CELLS – NEW PLAYERS IN SYSTEMIC SCLEROSIS CORRELATE WITH EXTENT OF SKIN AND LUNG FIBROSIS

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Introduction: Type 2 innate lymphoid cells (ILC2s), are recently identified as population of cells with lymphoid morphology lacking re-arranged antigen-specific receptors. Although findings in animal models of fibrotic diseases demonstrate increased numbers of ILC2s in fibrotic lesions, data on ILC2s in humans are mainly limited to allergic diseases. We aimed to evaluate the contributive role of ILC2s in the pathogenesis of systemic sclerosis (SSc), their levels and correlations with fibrotic manifestations in SSc.
Methods: Sixty-nine patients with SSc and 47 healthy controls were included into the study. Blood samples and skin sections were analyzed by flow cytometry and immunohistochemistry. ILC2 counts were correlated with clinical manifestations of SSc.
Results: Elevated numbers of ILC2s were detected in the skin (10-fold increase) as well as in the blood (4-fold increase) of SSc patients compared to healthy controls. As no single marker can sufficiently distinguish ILC2s from other cell population, we used two established sets of ILC2 markers to quantify ILC2 cells in the peripheral blood and the skin of SSc patients and healthy individuals, both of which yielded comparable results. Notably, activation markers are lacking on circulating ILC2s, however, after migration into the dermis ILC2s become activated as indicated by expression of IL-17RB and TSLP-R as well as by positive staining for the skin homing marker cutaneous lymphocyte antigen (CLA). Our data also suggest that ILC2s may be involved in the pathogenesis of fibrosis in SSc by showing multiple associations of ILC2 counts with fibrotic manifestations in SSc patients. Stratification of the SSc population in patients with limited (lcSSc) and diffuse cutaneous SSc (dcSSc) demonstrated increased levels of ILC2 in both subgroups with significantly higher frequencies in dcSSc compared to lcSSc. Moreover, dermal and circulating ILC2 counts correlated closely with the modified Rodnan skin score (mRSS). Increased ILC2 numbers were not only associated with more extensive skin fibrosis, but also with anti-topoisomerase antibodies and pulmonary fibrosis. ILC2 counts were highest in patients with extensive lung involvement assessed by CT scan.
Conclusions: Here, we provide first evidence for a role of ILC2s in the pathogenesis of rheumatic diseases by demonstrating increased ILC2 counts in the skin and blood of patients with SSc as compared to healthy individuals. Migration of circulating ILC2s into the skin, the activated state of dermal ILC2s and correlations of ILC2 counts with dermal and pulmonary fibrosis suggests a central role of ILC2s in the pathogenesis of fibrosis.

CO-23 | TIE2 AS A NOVEL FACTOR OF PERIPHERAL MICROANGIOPATHY IN SYSTEMIC SCLEROSIS

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Introduction: The angiopoietin (ang)/Tie2 system is a key regulator of vascular biology. The stable expression of membrane bound (mb) Tie2 and Ang1 is responsible for vessel stability, whereas Ang2, inducible by VEGF, hypoxia and pro-inflammatory stimuli, acts as an antagonist. The same applies to soluble Tie2 (sTie2), the extracellular domain of the Tie2 receptor, which is shedded by proteolytic cleavage upon stimulation with e.g. VEGF. Herein, we investigate the role of ang/Tie2 in the peripheral vasculopathy in SSc including different animal models.
Methods: The expression of Ang1/2 and Tie2 in the serum/dermis of SSc patients (n = 24/10) was compared with healthy donors (n = 20/8) by ELISA/immunohistochemistry (IHC). Next, expression levels of Ang/Tie2 were analysed in vascular (VEGF transgenic mice, n = 9) and non-vascular animal models of SSc (TSK1, bleomycin model; n = 4 each) including respective controls using immunohistochemistry.
Results: In skin biopsies of SSc patients, dermal microvessels abundantly expressed Ang-2, but not Ang-1, compared with healthy controls ($p = 0.004$). There was a profoundly decreased expression of mbTie2 receptor ($p<0.001$),

whereas the levels of soluble Tie2 (sTie2) were substantially increased (p = 0.001). Both in sera and skin of SSc patients, the Ang-1/2 ratio was reduced (p<0.001). These data point to a profound impairment of the ang/Tie-2 system in SSc favoring vessel destabilization. We next studied VEFG tg mice as vascular SSc animal model for Ang/Tie2 signalling. Interestingly, VEGF tg mice indeed showed the same dysregulation of Ang/Tie2 (p<0.05) as in humans, whereas non-vascular models such as TSK1 and bleomycin challenged mice did not fully mirror the human conditions.
Conclusions: Peripheral microvasculopathy in SSc results from a complex dysregulation of angiogenic signalling networks including VEGF and the Ang/Tie2 system. The Ang-/Tie-2 system is profoundly disturbed in SSc and might represent an important target for vascular therapeutic approaches.

CO-24 | THE ROLE OF IL-17 CYTOKINE FAMILY MEMBERS IN SCLERODERMA SKIN

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Introduction: A sparse inflammatory infiltrate rich in CD4+ T cells Is characteristically present in scleroderma (SSc) skin, particularly in perivascular areas. It is thought that CD4+ T cells may participate in the dysregulation of extracellular matrix deposition by directly or indirectly influencing fibroblast activities. We aimed at investigating the presence and function of cytokines mainly produced by Th17 cells, which numbers are increased in SSc.
Methods: Biopsies were obtained from the involved skin of 15 SSc, 4 morphea and 8 healthy donors (HD). Immunohistochemistry/immunofluorescence techniques were coupled to a semi-automated imaging quantification to determine the presence of the IL-17 family members+ and IL-22+ cells in the skin. The *in vitro* response of HD and SSc fibroblasts to IL-17 family members, IL-22, IL-22 in conjunction with TNF or keratinocyte conditioned medium was assessed by ELISA, RIA, real-time PCR and western blot. The *in vivo* response in mice was assessed by histo-morphometry.
Results: Positive cells for each of the IL-17 isoforms and IL-22 were present in the dermis of all the individuals tested. SSc individuals had increased frequency of IL-17A+ (p = 0.0237) and decreased frequency of IL-17F+ (p = 0.0127) and IL-17C+ cells (p = 0.0008) when compared to HD. IL-17E+ cells tended to be more frequent in SSc than in HD. Fibroblast production of IL-6, MMP-1 and MCP-1 was enhanced in a dose-dependent manner in the presence of IL-17E and IL-17F, but not in the presence of IL-17C. None of the cytokine tested had significant effect on type I collagen production. IL-22+ cells were over-represented in the epidermis of SSc compared to HD. Dermal fibroblasts expressed both IL-22 receptor subunits IL-10RB and IL-22RA, which expression was enhanced by TNF and reduced by TGF-beta. IL-22 induced rapid phosphorylation of p38 and ERK1/2 in fibroblasts, but failed to induce the synthesis of chemokines and extra-cellular matrix components. However, IL-22 enhanced the production of MCP-1, IL-8 and MMP-1 induced by TNF. Fibroblast responses were maximal in the presence of conditioned medium from keratinocytes activated by IL-22 in conjunction with TNF. Dermal thickness was maximal in mice injected simultaneously with IL-22 and TNF.
Conclusions: Dermal expression of IL-17C (low) and IL-17E (high) identifies a fibrosis-specific motif. IL-22 capacitates fibroblast responses to TNF and promotes a pro-inflammatory fibroblast phenotype by favoring TNF-induced keratinocyte activation. These results define a novel role for keratinocyte-fibroblast interactions in the context of skin fibrosis.

CO-25 | ESTROGENS INHIBIT THE PROFIBROTIC EFFECTS OF TGF-BETA AND PROTECT FROM THE DEVELOPMENT OF EXPERIMENTAL DERMAL FIBROSIS

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Introduction: Systemic sclerosis (SSc) primarily affects postmenopausal women. This sex bias raises the question of the role of female hormones on SSc phenotype. Our aim was to evaluate the effects of estrogens i) in the development of experimental dermal fibrosis and ii) on the response of dermal fibroblasts to transforming growth factor-β (TGF-β), the master cytokine of fibrosis.
Methods: Effects on estrogen inhibition by gene inactivation (knockout mice for the estrogen receptor-α, ERKOα) or targeted molecular strategy (tamoxifen, a selective estrogen receptor modulator that display anti-estrogenic properties) were evaluated in the mouse model of bleomycin-induced dermal fibrosis and in the tight skin (Tsk-1) mouse model.
SSc dermal fibroblasts were stimulated with TGF-β and incubated with different concentrations of 17-β-estradiol and/or tamoxifen. Collagen release from fibroblasts was evaluated by mRNA levels of col1a1 and col1a2 and by measuring the concentrations of collagen in cell culture supernatants with the SirCol collagen assay. Differentiation of fibroblasts into myofibroblasts was assessed by the expression of alpha smooth muscle actin (α-SMA). Activation of the TGF-β pathway was evaluated by the expression of phospho-smad-2/3.
Results: Estrogen inhibition increased the activation of canonical TGF-β signaling and exacerbated skin fibrosis both in the bleomycin model and in Tsk-1 mice. Upon bleomycin injections, ERKO-α mice treated with tamoxifen had a significant increase of dermal thickness (17%, p = 0.03 and 20%, p = 0.04), hydroxyproline content mice (16%, p = 0.02 and 36%, p = 0.003, respectively) and number of myofibroblasts (22%, p = 0.01 and 20%, p = 0.04 respectively) compared to control mice. In Tsk-1 mice, treatment with tamoxifen led to significantly enhanced skin fibrosis, with a 31 ± 8% increase of hypodermal thickening (p = 0.03) and a 17% increase of hydroxyproline content (p = 0.01) compared to control mice.
In SSc dermal fibroblasts, treatment with 17-β-estradiol significantly decreased the stimulatory effects of TGF-β on collagen synthesis and myofibroblast differentiation, decreased activation of canonical TGF-β signaling, and markedly reduced the expression of TGF-β target genes. Tamoxifen reversed the inhibitory effects of estrogens by restoring Smad2/3 phosphorylation and TGF-β-induced collagen synthesis.
Conclusions: Our results demonstrate a beneficial effect of estrogen in experimental dermal fibrosis. Estrogens reduce TGF-β dependent activation of SSc dermal fibroblasts and estrogen inhibition leads to a more severe experimental dermal fibrosis. These findings may partly contribute to the occurrence of SSc in postmenopausal women and the greater severity of the disease in men and open avenue to potential hormonal therapies.

▶ Lecture 3

CO-26 | INCREASED FREQUENCY OF MALIGNANCIES, AND IN PARTICULAR BREAST CANCER, SYNCHRONOUS TO THE ONSET OF SYSTEMIC SCLEROSIS IN ANTI-RNA POLYMERASE III ANTIBODIES POSITIVE PATIENTS: A EUSTAR MULTICENTRE STUDY

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Introduction: The relatively low frequency of anti-RNA polymerase III antibodies (anti-RNAP) in systemic sclerosis (SSc) and the disease heterogeneity limit the possibility to accurately define the phenotype of SSc patients with anti-RNAP. Determining the clinical manifestation associated with anti-RNAP is nevertheless critical, taking into account recent data that demonstrated a link between immune response against cancer cells and the generation of anti-RNAP. However, most data on clinical associations of anti-RNAP derived from single centre-studies, or relatively small case series. In the present study, to overcome these issues, we used the large multinational cohort of the EULAR Scleroderma Trials And Research (EUSTAR).
Methods: The EUSTAR database was searched: patients from with information on anti-RNAP status were avaialable, were included in the analysis. Moreover, centers were then asked to participate to a case-control study providing data not recorded in the EUSTAR database. Anti-RNAP+ patients

were compared with anti-RNAP-negative SSc controls, matched for sex, cutaneous subset and disease duration, and age at disease onset.
Results: Anti-RNAP status was available in 4,986 of 11,399 patients from EUSTAR database: 223 were anti-RNAP+. After exclusion of 47 patients with multiple autoantibody positivity, 176 anti-RNAP+ patients were compared with 4,763 anti-RNAP-negative patients: multivariate analysis confirmed the association of anti-RNAP with scleroderma renal crisis (p<0.0001, OR 7.06; 95% CI 3.77 to 12.2) and diffuse cutaneous involvement (p<0.0001, OR 2.35; 95% CI 1.58 to 3.49).
In the case-control study 158 anti-RNAP+ and 199 anti-RNAP-negative patients were collected from 13 EUSTAR centers. We found an association of anti-RNAP with renal crisis (p:0.0005, OR 5.30; 95% CI 1.81 to 16.6), gastric antral vascular ectasia (p:0.0009, OR 8.80; 95% CI 1.85 to 57.4) and a shorter time to reach the peak of mRSS (p:0.013, log-rank test). An increased rate of malignancies was observed (OR 2.17; 95% CI 1.15 to 4.08). This was accounted for by the increased rate of tumors synchronous to the onset of SSc (OR 7.38; 95% CI 1.61 to 33.8). In particular, risk of breast cancer synchronous to the onset of SSc was increased (OR 20.2; 95% CI 1.41 to 355).
Conclusions: Anti-RNAP are associated with severe clinical manifestations of SSc, and with malignancies synchronous to the onset of SSc (in particular, breast cancer). These findings have relevant prognostic implications which should guide the monitoring and follow-up of these patients.

▶ Lecture 4

CO-27 | IMPAIRED MICRONUTRIENT STATUS IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Introduction: Micronutrients are essential dietary factors involved in numerous metabolic processes, including oxidative stress, collagen synthesis and wound healing, which are also important for the pathogenesis of systemic sclerosis (SSc). Considering the frequent gastrointestinal involvement and impaired nutritional status, we hypothesized that the micronutrients could be profoundly affected in SSc patients.
Methods: Patients meeting the ACR/EULAR 2013 classification criteria for SSc were prospectively recruited between 2009 and 2014. Clinical assessment, data recording and quality controls were done according to EUSTAR standards. In addition, the UCLA SCTC-GIT 2.0 questionnaire was applied and the circulating levels of the following micronutrients were measured: zinc, selenium, prealbumin, holotranscobalamin, vitamin B12, folic acid, red cell folate. Patients with single or multiple micronutrient deficiency (-ies) were compared to patients with a normal micronutrient pattern. The two-sided Fisher's exact test, double T-test and the Mann-Whitney U-test were used, as appropriate. Binary logistic regression was applied to identify risk factors for deficiency in micronutrients.
Results: Out of the 176 patients with SSc included into the study, almost half (44%) showed a deficiency in at least one of the measured micronutrients. The most frequent deficit was seen in selenium (22% of patients), followed by folic acid (17%) and prealbumin (15%). Nearly a fifth (19%) of these patients had multiple deficiencies. There was a significant association between low levels of zinc and selenium, prealbumin and folic acid, respectively. Patients with lower body mass index (BMI) had significantly lower zinc levels, and those with low prealbumin had more frequent stomach symptoms. Advanced skin fibrosis (higher modified Rodnan skin score, p = 0.007; skin thickening proximal to the metacarpophalangeal (MCP) joints, p = 0.009; positive ACR 1980 classification criteria, p = 0.014), as well as lower hemoglobin (Hb) levels (p<0.001), were strongly associated with deficiency in micronutrients. In the predictive model, low Hb, low BMI and skin thickening proximal to MCP were confirmed as risk factors for deficiency in micronutrients, the latter being associated with a nearly 5-fold increased risk (p = 0.037, OR 4.96, 95% CI [1.1-22.4]).
Conclusions: These novel data reveal that deficiencies in micronutrients are a frequent and often complex burden in patients with SSc. Moreover, these correlate with clinical aspects of the disease. Especially patients with more advanced skin fibrosis are at high risk for an impaired micronutrient status. These data have potential clinical implications, as they suggest that screening for micronutrients status should be performed in these patients.

Session 8 | Round table SSc registries in the world

CO-28 | PHENOTYPES DETERMINED BY CLUSTER ANALYSIS AND THEIR SURVIVAL IN THE PROSPECTIVE EUSTAR COHORT OF PATIENTS WITH SYSTEMIC SCLEROSIS

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Introduction: Systemic sclerosis (SSc) is classically dichotomized in limited or diffuse cutaneous subsets (lcSSc/dcSSc) associated with different prognosis. However, it appears that SSc is a highly heterogeneous disease. The primary objective of our study was to identify and characterize homogeneous phenotypes in the EUSTAR SSc population using a cluster analysis. The secondary objective is to assess the survival in the different clusters.
Methods: The participants were SSc patients of the EUSTAR prospective cohort. Inclusion criteria were: adult patients, fulfilled American College of Rheumatology criteria for SSc, and had a calculable follow up. Hierarchical clustering based on the Ward method was performed using 25 clinically meaningful variables. The clinical relevance of the clusters was analysed by their characteristics and survival outcomes. Survival curves were plotted by the Kaplan-Meier method. Crud and adjusted (on the age at diagnosis and gender) Cox models were performed.
Results: A total of 6,927 patients were analyzed. Cluster analysis identified 5 clusters. The first cluster C1 (n = 1,963) was mainly characterized by a low mean Rodnan skin score (mRSS) contrasting with a relatively similar percentage of anti-topoisomerase 1 (ATA) and anti-centromere (ACA) (28% and 40%) and a high percentage of lung fibrosis (48%); the second cluster C2 (n = 1,186) by a large majority of lcSSc (89%) and ACA (79%) and the lowest percentage of lung fibrosis (22%); the third cluster C3 (n = 1,249) and the fourth cluster C4 (n = 856) by a high percentage of dcSSc/ATA (72%/50% and 92%/77% respectively) and the highest proportion of male patients. Digital ulcers, renal crisis/anti RNA polymerase III antibodies, joint involvement were more frequently found in C4 than in the other clusters. Patients in the cluster C5 (n = 1,673) were mainly female patients, older than in the other clusters, had a rather low mRSS, a high percentage of ATA (46%) and a high percentage of lung fibrosis. Concerning survival, C2 had the best survival. When compared to C1, C2 to C5 clusters had a significantly different prognosis (C2: adjusted hazard ratio [95% CI]: 0.70 [0.51-0.96]; C3: 2.27 [1.76-2.94]; C4: 4.52 [3.57-5.72]; and C5: 1.94 [1.51-2.44].
Conclusions: This cluster analysis on the largest ever worldwide database of SSc patients showed 5 different clusters characterized by different sex ratio, mRSS, autoantibodies status, joint and organ involvement as well as by a different prognosis. Although systemic sclerosis is a heterogeneous disease, this study shows that homogeneous groups beyond the classical limited/diffuse dichotomy can be delineated in this disease.

CO-29 | LOW FREQUENCY OF RENAL CRISIS IN MORE THAN 3100 PATIENTS OF THE GERMAN NETWORK FOR SYSTEMIC SCLERODERMA (DNSS)

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Background: Scleroderma renal crisis (SRC) is a rare manifestation but still a medical emergency in patients with SSc. To improve detection and follow-up of patients with SSc, the German Network for Systemic Scleroderma (DNSS) was founded 2003 implementing a patient registry recording data on diagnosis, organ involvement and therapy in a prospective manner.
Methods: Up to date, more than 3000 patients have been grouped into four descriptive disease subsets, i.e. limited cutaneous disease (lcSSc), diffuse cutaneous disease (dcSSc), overlap-syndrome and undifferentiated connective tissue disease (UCTD) with scleroderma features.
Objectives: Within this analysis we have focused on SRC within the three main subsets, e.g. lcSSc, dcSSc and SSc overlap-syndromes, to identify baseline aspects, which are associated with a higher risk for future SSc-associated renal crisis.
Results: Recent analyses of up to now 3180 patients revealed that 56% of patients suffer from limited SSc (lcSSc), 34% from diffuse SSc (dcSSc) and 11% of patients were diagnosed with an overlap-syndrome. Eighteen patients developed a renal crisis (1.4%, 18/3180), while 10% (315/3180) were classified with kidney involvement and 8% (257/3180) with proteinuria. Of these, 66.7% (12/18) were diagnosed with the diffuse form of SSc, while just 27.8% (5/18) were diagnosed with lcSSc and 5.6% (1/18) with SSc-overlap syndromes. Predictive factors for renal crisis in our patient cohort included the positive anti-RNA polymerase (RNAP) autoantibodies (OR 24.6, p<0.0001, 95%-CI 6.1-99.5), proteinuria (OR 11.8, p<0.0001, 95%-CI 4.3-32.1), hypertension (OR 6.1, p<0.0001, 95%-CI 2.3-16.5), tendon friction rubs (OR 5.4, p = 0.004, 95%-CI 1.7-16.9), elevated CK-levels (OR 5.1, p = 0.01, 95%-CI 1.4-18.9), a modified Rodnan skin score (mRSS) of more than 15 (OR 4.7; p = 0.002, 95%-CI 1.7-13) and diffuse form of SSc (odds ratio (OR) 4.6, p = 0.005, 95%-confidence interval (CI) 1.6-13.5). Patients diagnosed with renal crisis were significantly more frequent on ACE-inhibitors (61.1%, 11/18, p = 0.001). Of these, 5 patients also suffered from proteinuria and 7 patients from hypertension. Patients on systemic glucocorticoids had also an increased risk to develop a renal crisis (OR 5.1, p = 0.002, 95%-CI 1.8-14.3), independent of the dosage (>or <7.5 mg/day).
Conclusions: Compared to data collected 10 to 20 years ago, SRC has become a rare complication in SSc with a frequency of less than 2%.
Whereas a number of previously defined risk factors were confirmed, it remains to be shown which factors are protective for the development of SRC.

CO-30 | GUIDELINES FOR THE RATIONAL USE OF FOLLOW-UP CARDIAC ECHOCARDIOGRAPHY TO SCREEN FOR PULMONARY ARTERIAL HYPERTENSION (PAH) IN SYSTEMIC SCLEROSIS (SSC)

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Introduction: Clinical practice guidelines recommend screening of all systemic sclerosis (SSc) patients for pulmonary arterial hypertension (PAH) with yearly echocardiograms. However, this may not be cost effective due to its low sensitivity and specificity and the low incidence of PAH in SSc. The primary objective of this study was to develop a risk prediction score to identify SSc patients who do not need an annual echocardiogram after a baseline echocardiogram.
Methods: Data were extracted from the Canadian Scleroderma Research Group registry. PAH was defined as a resting mean pulmonary arterial pressure of ≥25 mmHg and a pulmonary capillary wedge pressure of ≤15 mmHg, confirmed by right heart catheterisation. Univariate analysis was used to identify clinical variables associated with incident PAH. Multivariate logistic regression was used to identify independent predictors among selected variables (p<0.05 in univariate analysis). Backward selection was performed to identify the most parsimonious model. The performance characteristics of the models were estimated using sensitivity, specificity, area under the receiver operating characteristics curve (AUC), and coefficient of determination (R^2). The estimated probability of incident PAH for all subjects was calculated using parameters from the backward selection model. We identified a cutoff of the estimated probability of incident PAH below which no subject had PAH.
Results: This study included 1034 patients (87% female, mean (SD) age 57.5 (11.8) years and disease duration from first non-Raynaud's disease symptom 13.2 (9.2) years, 45% with diffuse cutaneous SSc, and 37 (3.6%) with incident PAH. Shortness of breath (SOB) and diffusing capacity for carbon monoxide (DLCO) were the only independent predictors of incident PAH in multivariate analysis. The sensitivity, specificity, AUC and R^2 of the full model were 90.0%, 56.3%, 90.3% and 28.8%, respectively, and of the selection model 90.9%, 60.3%, 88.3% and 23.9%, respectively. All cases of PAH had an estimated probability of incident PAH >1.6%, calculated using parameters from the backward selection model. There were 56.6% of the subjects who had an estimated probability of PAH <1.6%, of which none had PAH.
Conclusions: A simple risk prediction score consisting of DLCO and SOB can identify subjects at risk of PAH in a general SSc population. More than 50% of SSc subjects are at very low risk of PAH and it would be reasonable to defer annual echocardiogram screening in this group, representing large savings in health expenditures. These findings represent the first evidence-based risk score for the rational use of echocardiograms in an unselected SSc cohort.

CO-31 | DETERMINANTS OF UNEMPLOYMENT AMONGST AUSTRALIAN SCLERODERMA PATIENTS: RESULTS FROM A LARGE MULTICENTRE COHORT STUDY

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Introduction: Work disability is a significant consequence of chronic rheumatic disorders, impacting both the society through indirect costs and the individual and their family through loss of income and social activities, and a negative influence on quality of life. We sought to assess employment status, risk factors for unemployment, and the associations of unemployment with patients' health related quality of life (HRQoL).
Methods: Patients enrolled in a SSc longitudinal observational cohort were included. All patients completed an employment questionnaire on enrollment. Clinical manifestations were defined as present if present at the time of enrollment. Pulmonary arterial hypertension (PAH) was diagnosed on right heart catheterization (mPAP >25 and PAWP <15 mmHg). Summary statistics,

chi-square tests, univariate and multivariable logistic regression were used to determine the associations of different risk factors with employment. All statistical analysis were performed using STATA 14.0.
Results: Among 1587 SSc patients, 160 (20%) were unemployed at the time of cohort enrolment. Of these 82.5% were female, 63% had limited disease subtype. Mean (± SD) age and disease duration at recruitment from first non-Raynaud's symptom were 51.9 (± 10.4) and 11.1 (± 10.9) years, respectively. Multivariable regression analysis revealed the presence of digital amputation (OR 3.9, 95% CI 1.7-9.1, p = 0.002) diffuse disease subtype (OR 2.2, 95% CI 1.3-3.5, p-value = 0.002), sicca symptoms (OR 2.7, 95% CI 1.6-4.4, p<0.001), physical job (OR 1.8, 95% CI 1.1-3.1, p = 0.03) and PAH (OR 2.2, 95% CI 1.1-4.5, p = 0.02) to be associated with unemployment. Unemployed patients had consistently poorer HRQoL scores in all domains (physical, emotional and mental health) of the SF-36 form than those who were employed.
Conclusions: SSc is associated with substantial work disability and poor quality of life. Raising awareness, identifying modifiable risk factors and implementing employment strategies and modifications to work place environments are possible ways of reducing this burden and potentially improving patients' HRQOL.

▶ Lecture 5

CO-32 | SURVIVAL AND ORGAN INVOLVEMENT IN PATIENTS WITH LIMITED CUTANEOUS SYSTEMIC SCLEROSIS AND ANTI-TOPOISOMERASE ANTIBODIES: MORE LIKE LIMITED CUTANEOUS OR MORE LIKE DIFFUSE CUTANEOUS SYSTEMIC SCLEROSIS?

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Introduction: Systemic sclerosis (SSc) has two main subtypes based on skin involvement: limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc). LcSSc is associated with anti-centromere antibodies (ACA) and a mild course whereas dcSSc is associated with anti-topoisomerase antibodies (ATA) and a severe course. However, ATA can also be present in lcSSc, but little is known about this subgroup and the course of disease concerning survival and organ involvement. If it is possible to improve subclassification of SSc patients and to improve recognition of high risk patients, those patients can be screened more tightly and receive treatment to prevent organ involvement and premature death.
The objective is to determine whether survival and organ involvement of lcSSc ATA positive patients resemble lcSSc ATA negative patients or dcSSc ATA positive patients. Furthermore, time to transition from lcSSc to dcSSc was evaluated in ATA positive and ATA negative patients.
Methods: Data from The Nijmegen Systemic Sclerosis cohort were used with up to 15 years of follow-up. Kaplan-Meier analysis was performed to show the cumulative survival and organ involvement rates including; Interstitial Lung Disease (ILD), Pulmonary arterial hypertension (PAH), cardiac involvement and Scleroderma Renal Crisis (SRC). Subgroups were formed according to subtype (lcSSc or dcSSc) and ATA (positive or negative). A Cox proportional hazard model was performed to adjust for confounders.
Results: There were 460 patients included, 58 (12.6%) lcSSc ATA positive subjects, 237 (51.5%) lcSSc ATA negative subjects, 78 (17.0%) dcSSc ATA positive subjects and 87 (18.9%) dcSSc ATA negative subjects. Survival during 15 years of follow up was highest in lcSSc ATA positive subjects with a cumulative survival of 75.1%, whereas dcSSc ATA positive subjects had lowest survival, with a cumulative survival of 52.9% (p = 0.012). The largest difference in organ involvement was in ILD. LcSSc ATA positive subjects had a higher occurrence of ILD than lcSSc ATA negative subjects, and a lower occurrence of ILD then dcSSc ATA positive subjects.
Forty-eight lcSSc patients developed dcSSc, 24 (9.2%) in the ATA negative subset and 24 (29.3%) in the ATA positive subset.
Conclusions: lcSSc ATA positive subjects do not resemble lcSSc ATA negative subjects or dcSSc ATA positive subjects, but have a different course of disease regarding survival and organ involvement. Subtyping based on skin involvement may be incomplete. This could be the first step in development of risk stratification for lcSSc ATA positive patients and the development of a new subclassification for systemic sclerosis patients.

Parallel session 9 | Experimental models of scleroderma

CO-33 | UPAR-DEFICIENT MICE: A NOVEL EXPERIMENTAL MODEL OF DELAYED CUTANEOUS WOUND HEALING TO EXPLORE SSC-RELATED DIGITAL ULCERS

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Introduction: Digital ulcers are a painful complication of systemic sclerosis (SSc). Their treatment is challenging, and therapeutic options are limited. The development of animal models of SSc-related ulcers would help to screen drugs with potential benefit. Urokinase-type plasminogen activator receptor (uPAR) knockout mice was recently described as a good model of peripheral microvasculopathy and skin fibrosis. The objective of this work was to assess the wound healing process on uPAR-KO mice in comparison with uPAR-wt mice.
Methods: Eight adult uPAR-wt and eight adult uPAR-KO mice were anesthetized with isoflurane and shaved. One full-thickness skin excision wound was realized with a biopsy punch of 6-mm diameter on the dorsal midline on each animal. All mice were subsequently equipped with silicone rings centered and fixed around the wound to assess the impact of wound contraction (phenomenon observed in rodents) on the healing process. Daily assessment of wound healing was performed by taking pictures analyzed with computer. Repeated-measure ANOVAs were performed to compare the wound healing delay between uPAR-wt and uPAR-KO mice.
Results: uPAR-KO mice showed delayed wound healing process in comparison with uPAR-wt mice (p = 0.007). The largest difference in the percentage of healing bewtween uPAR-KO and uPAR-wt mice was observed after four days of healing (52.5% vs 25.1% respectively, p = 0.005). Animals from both groups showed a complete
Conclusions: SS-related digital ulcers are very handicapping complications and lead to a decrease in the quality of life of patients. Here we showed that uPAR-KO mice presented a delayed wound healing process, making this model a good model to explore new pharmacological strategies in the treatment of these ulcers. Futher experiments with this novel experimental model should be proposed to screen potential drugs that would enhance the wound healing process.

CO-34 | GLI2 AS A TARGET FOR ANTIFIBROTIC THERAPIES IN PRECLINICAL MODELS OF SSC

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Introduction: Hedgehog signaling plays a crucial role in the pathogenesis of Systemic sclerosis (SSc). Besides canonical hedgehog signaling with Smoothened (Smo)-dependent activation of Gli transcription factors, Gli can be activated independently of classical hedgehog ligands and receptors (non-canonical pathways). The aim of the present study was to characterize the role of non-canonical hedgehog signaling in SSc and to address the efficacy of direct Gli-inhibitors that target simultaneously canonical and non-canonical hedgehog pathways.
Methods: Canonical and non-canonical hedgehog signalings were simultaneously inhibited using the Gli-inhibitor GANT-61. Canonical hedgehog signaling was selectively targeted with the Smo-inhibitor vismodegib. In addition, Gli2 was selectively depleted in fibroblasts taking advantage of Gli2fl/fl and Col1a2CreER mice. The effects of pharmacologic or genetic of Gli2 on TGF-βsignaling were analyzed in cultured fibroblasts, in bleomycin-induced pulmonary fibrosis and in mice with overexpression of a constitutively active TGF-βreceptor I (TBRI).
Results: The expression of Gli2 was transcriptionally upregulated in fibroblasts and in murine tissues by TGF-β in a Smad3-independent manner. Fibroblast-specific knockout of Gli2 ameliorated TBR-induced skin fibrosis. Consistent a novel role of Gli2 as a downstream mediator of TGF-β in fibroblasts, inhibition

of Gli2 by GANT-61 ameliorated the stimulatory effects of TGF-β on fibroblasts *in vitro* and *in vivo*. Treatment with GANT-61 downregulated the levels of TGF-β targeted genes, reduced the collagen release and blocked TGF-β-induced myofibroblast differentiation. In contrast, inhibition of canonical hedgehog signaling by vismodegib did not ameliorate TGF-βinduced fibroblast activation. In the mouse model of TBRI-induced fibrosis, mice treated with GANT-61 showed reduced dermal thickening, lower myofibroblast counts and decreased hydroxyproline content, whereas inhibition of canonical hedgehog signaling by vismodegib had no anti-fibrotic effect. GANT-61 also exerted potent anti-fibrotic effects in a model of pre-established bleomycin-induced pulmonary fibrosis. In both models, GANT-61 did not only reduce levels of the hedgehog target genes, but also the levels of the TGF-βtarget genes, thus confirming inhibition of TGF-β signaling upon targeting of Gli2.
Conclusions: These results characterize Gli2 as a central mediator of fibroblast activation in SSc and as a potential target for anti-fibrotic therapies. We demonstrate that Gli2 is activated in SSc not only by canonical, but also by TGF-β dependent, non-canonical pathways. Genetic and pharmacologic inhibition of Gli2 ameliorates fibrosis in murine models of dermal and pulmonary fibrosis in preventive and therapeutic settings. These findings may have translational implications as non-selective inhibitors of Gli2 are already in clinical use and selective inhibitors are currently developed.

CO-35 | SMALL PEPTIDES DERIVED FROM MET RECEPTOR TYROSINE KINASE AS NEW THERAPEUTIC APPROACH FOR THE TREATMENT OF SCLERODERMA

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Introduction: Hepatocyte growth factor receptor, also known as cellular mesenchymal-epithelial transition factor (c-MET, MET), is an important antifibrotic molecule that protects various tissues, including lung, from injury and fibrosis. The intracellular cytoplasmic tail of MET contains several motifs demonstrating antifibrotic effects in lung and skin fibroblasts.
Methods: Lung fibroblasts were derived from lung tissues obtained at autopsy. Skin fibroblasts were isolated from the biopsy samples obtained from the involved forearm skin of SSc patients and from age-, sex-, and race-matched healthy adult donors. Human alveolar epithelial cells (AEC) were purchased from ScienCellonline. Antifibrotic *in vivo* effects of M10 peptide (10 mg/kg, intraperitoneal, every 48h) were studied in the bleomycin murine model of scleroderma lung disease. Potential peptide-protein interactions were modulated in-silico and investigated by co-immunoprecipitation and protein interaction assays. Protein localization, expression, and phosphorylation were determined by immunoblotting, immunohistochemistry, and immunofluorescent studies.
Results: MET-derived peptides, when added to cell culture medium, remain in the cytoplasm and nuclei of cells for up to 24 hours. The peptides effectively decrease collagen, connective tissue growth factor (CTGF, CCN-2), and smooth muscle α-actin (SMA) in both scleroderma and TGFβ-stimulated normal lung and skin fibroblasts. MET-derived peptides interact with the MH2 domain of Smad2 and inhibit TGFβ-induced Smad2 phosphorylation. 1403 and 1404 peptides directly bind caspase-3 and protect AEC from FasL- and Cisplatin-induced apoptosis. In the bleomycin murine model of pulmonary fibrosis, M10 noticeably reduces fibrotic lung parenchyma. A semi-quantitative evaluation of histopathology by Ashcroft scale demonstrates a substantial decrease in bleomycin-induced fibrosis of M10-treated mice as compared to mice treated with scrambled peptide. In fact, the fibrotic score in mice that received bleomycin and scrambled peptide was 8.2-fold higher compared to control (saline-treated) mice (5.63 ± 1.72 and 0.69 ± 0.35, respectively). The score in mice that received bleomycin and were treated with M10 was reduced to 1.67 ± 1.01, reflecting a pronounced antifibrotic effect of M10.
Conclusions: MET-derived peptides demonstrate antifibrotic effects by counteracting Smad-dependent fibrogenic pathways. Cell-protective effects of MET-derived peptides 1403 and 1404 in AEC are based on inhibition of caspase-3-induced apoptosis. MET-derived peptides should be considered as potential therapeutic agents for systemic sclerosis and other fibrosing diseases.

CO-36 | CM-101, A NOVEL MONOCLONAL ANTIBODY BLOCKING CCL24 AMELIORATES EXPERIMENTAL SYSTEMIC SCLEROSIS (SSC) AND IDIOPATHIC PULMONARY FIBROSIS (IPF)

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Introduction: SSc and IPF share in part, common pathogenetic pathways in the genesis of tissue fibrosis. Chemokines have been sporadically reported to be expressed in these disorders. Previously, we have shown that the CCL24/CCR3 pathway is significantly expressed in affected SSc skin tissues and in the lungs of IPF patients. A novel monoclonal antibody (mAb) against human CCL24 was shown to attenuate chemokine induced fibroblast and inflammatory cell migration *in vitro*. Aim: to test CM-101 in two models of SSc and IPF induced either by subcutaneous or intratracheal instillation of bleomycin (BLM).
Methods: C3H and C57bl/6J mice were employed for both models respectively. Each group comprised of 9-12 animals. The SSc model was induced by repeated injections of BLM whereas the IPF model was induced by a single intratracheal instillation of BLM. CM-101 was developed against human CCL24, yet displayed significant cross reactivity to murine CCL24.
Results: CM-101 as compared to control IgG delivered intraperitoneally prior to repeated subcutaneous injections of BLM, significantly reduced collagen deposition and dermal thickness in the SSc model. CM-101 as compared to control IgG given IP prior to BLM instillation in the IPF model, significantly reduced inflammatory cell counts in the BAL fluid ($p<0.01$). Lung collagen content evaluated by the Sircol assay was reduced by CM-101 as compared to control IgG ($p<0.05$) and this finding was corroborated by histological analysis of lung sections showing attenuated fibrotic area ($p<0.05$). When CM-101 was administered 7 days after BLM instillation, when evidence of lung damage was already evident, it also attenuated lung fibrosis as compared to IgG control ($p<0.05$).
Conclusions: CM-101, a novel mAb blocking CCL24 is effective in reducing skin thickness and lung fibrosis in two murine models of experimental SSc and IPF, respectively. These findings may merit further investigation and potential application of this new monoclonal antibody in the clinic.

CO-37 | A NOVEL ANTIBODY BLOCKING CCL24/CCR3 REDUCES CHEMOKINESIS OF IMMUNE CELLS AND THE TRANSITION OF FIBROBLASTS TO MYOFIBROBLASTS IN SYSTEMIC SCLEROSIS (SSC)

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Introduction: In SSc the contribution of inflammation, fibrosis and endothelial dysfunction is significant to the evolution of the disease. Chemokines have been shown to be involved in this process and some have been circumstantially associated with the pathogenesis of SSc. Usually, CCL24 and its receptor CCR3 are potent chemokines that are principally investigated in allergic reactions. Aim: to perform a preliminary evaluation of the role of the CCL24/CCR3 pathway in SSc either *in vivo* and *in vitro* and verify the role of a new monoclonal antibody against CCL24 in interfering with some fundamental mechanisms of SSc pathogenesis.
Methods: Sera were obtained from 21 patients with SSc and age/sex matched controls. Levels of CCL24, Eotaxin-1 and RANTES were assessed by ELISA. Affected skin biopsies were obtained from SSc patients (n = 8) and studied by immunohistochemistry using commercial mouse anti human anti-CCL24 antibody. The Monoclonal CM-101 was produced by ChemomAb ltd in mice by repeated immunization with human CCL24 and subsequently humanized by CDR grafting.
Results: Immunohistochemical staining of SSc skins exhibited significant expression of CCL24 and CCR3 in inflammatory and endothelial cells, respectively. No expression was evident in healthy skin tissues. Serum levels of CCL24 were significantly increased in patients with SSc (median 320 pg/ml) as compared to age matched controls (120 pg/ml; $p<0.001$). CM-101, a humanized monoclonal antibody to CCL24, significantly attenuated chemokine-triggered migration of SSc sorted T cells/monocytes as compared to control IgG ($p<0.01$). Moreover, CM-101 was also capable of attenuating chemokine induced activation of CCR3 expressing cell lines evidenced by calcium release assays. *In vitro*, the SSc sera triggered transition of human fibroblasts to myofibroblasts that was strongly suppressed by preincubation with CM-101 as compared to control IgG ($p<0.01$).

Conclusions: CCL24 levels are significantly higher in SSc sera and this chemokine is also expressed along with its receptor in affected SSc skin tissues. *In vitro*, a humanized blocking antibody to the CCL24 attenuates chemokinesis of immune cells and reduces fibroblast to myofibroblast transition. This novel monoclonal antibody might represent a new therapeutic strategy for SSc patients.

CO-38 | EFFECTS OF NINTEDANIB ON FIBROTIC AND VASCULAR MANIFESTATIONS IN PRECLINICAL MODELS OF SYSTEMIC SCLEROSIS

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Introduction: Nintedanib is a powerful inhibitor that inhibits PDGF-, FGFR-, VEGFR-receptors and Src kinases activity simultaneously. It has been recently approved for the treatment of idiopathic pulmonary fibrosis (IPF). The aim of this study was to evaluate the effects of nintedanib on fibrotic manifestations in preclinical models of systemic sclerosis (SSc) as well as on vascular manifestations in the fos-related-antigen-2 (Fra2) model.
Methods: The effects of nintedanib on migration, proliferation, myofibroblast differentiation and collagen release of cultured human dermal fibroblasts were investigated by MTT- and scratch assays, stress fiber staining, qPCR and SirCol assays. In addition, nintedanib was evaluated in bleomycin-induced skin fibrosis, in murine sclerodermatous cGvHD, in Tsk-1 mice and in Fra2-tg mice.
Results: Nintedanib inhibited the PDGF- and TGFbeta-induced fibroblast proliferation and migration more effectively as compared to selective inhibition of PDGF-, VEGF or FGF-receptors. Nintedanib dose-dependently reduced the mRNA levels of Col 1a1, Col 1a2 and fibronectin-1, reduced collagen release and inhibited myofibroblast differentiation in healthy fibroblasts stimulated with TGFbeta or PDGF. In SSc fibroblasts, nintedanib reduced the endogenous activation and decreased the collagen release even without exogenous stimulation. Nintedanib exerted potent anti-fibrotic effects in bleomycin-induced skin fibrosis, in experimental cGvHD, in Tsk-1 and in Fra2-tg mice with dose-dependent amelioration of dermal and pulmonary fibrosis. Treatment with nintedanib also inhibited vascular manifestations in Fra2-tg mice. Proliferation of vascular smooth muscle cells with subsequent thickening of the vessel walls and luminal occlusion of pulmonary arteries was reduced in mice treated with nintedanib. Treatment with nintedanib also inhibited apoptosis of dermal microvascular endothelial cells and blunted the capillary loss in Fra2-transgenic mice.
Conclusions: Nintedanib inhibits fibroblasts activation and exerts anti-fibrotic effects in several complementary mouse models of SSc. Nintedanib also ameliorates SSc-like vascular features in Fra2-tg mice. These preclinical findings might have direct implications for the upcoming phase III clinical trial with nintedanib in SSc.

CO-39 | M2-SHIFTED MICE RECAPITULATE DERMAL AND PULMONARY REMODELING

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Introduction: Characteristics of systemic sclerosis (SSc) includes excessive fibrosis and microvascular abnormalities. Several lines of recent evidence showed that monocytes were actively involved in pathogenic process of SSc through differentiating into alternatively activated (M2) rather than classically activated (M1) phenotype. We accidentally found that mice transgenic (TG) for Fra-1, one of AP-1 components, developed thickening of lung interstitium and narrowing of the lumen of pulmonary arteries. Interestingly, it has been reported that Fra-1 TG mice represent resistance to dextran sulfate sodium-induced colitis by suppression of monocyte differentiation into M1 phenotype. The aim is to investigate whether M2-shifted monocytes promote fibrotic phenotype using Fra-1 TG mice.
Methods: Eleven pairs of Fra-1 TG mice and sex and age-matched control wild-type mice were compared. Infiltrating immune cells and phenotypes of monocytes in lung tissues were examined by immunofluorescence. Influence of Fra-1 expression in the phenotypic alteration of monocytes was assessed by expression of genes associated with M1 or M2 phenotypes using semi-quantitative PCR after *in vitro* culture of bone marrow-derived mononuclear cells. Remodeling of parenchyma or vessels in lungs and of skin was assessed using Masson-Trichrome, or Elastica-van Gieson staining. Extent of pulmonary fibrosis, pulmonary hypertension, and skin thickening were quantified by Ashcroft score, velocity of tricuspid regurgitation (TR) jet estimated by transthoracic echocardiography, and the thickness of dermis, respectively.
Results: In the lung tissues, perivascular infiltration of inflammatory cells was already seen at week 5 after birth, which was preceded to the remodeling. Monocytes were infiltrated dominantly (40 ± 10 vs 21 ± 7/HPF, P<0.05) and M2/M1 ratio was higher in the TG mice (4.7 ± 0.9 versus 0.2 ± 0.1, P<0.05). Upregulated expression of Fra-1 in monocytes significantly induced expression of M2-related genes such as IL-10 and arginase-1 (P<0.02, P<0.05 respectively). As for the remodeling, extent of pulmonary fibrosis was progressed in the TG mice compared with controls (5.3 ± 2.3 vs 0 ± 0, P<0.0008). Also, the TG mice showed concentric laminar intimal fibrosis of pulmonary arteries and had significant TR (331 ± 134 vs 113 ± 30 mm/s, P<0.05). Skin thickening was also observed in the TG mice (216 ± 26 vs 118 ± 22 μm, P<0.003).
Conclusions: M2-shifted environment in Fra-1 TG mice promote dermal and pulmonary remodeling, resembling histologically to skin sclerosis, interstitial lung disease, and pulmonary arterial hypertension typically observed in patients with SSc.

CO-40 | NOX4 IS A CRUCIAL MEDIATOR OF TGF-BETA1-INDUCED FIBROBLAST ACTIVATION OF DERMAL FIBROBLASTS – NOVEL FINDINGS FROM *IN VITRO* AND *IN VIVO* SCLERODERMA RESEARCH

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Introduction: Members of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) family are emerging as important players in a number of fibrotic diseases. We recently hypothesized that Nox4 could be a promising target for the treatment of systemic sclerosis (SSc) (Böhm et al., Exp. Dermatol. 2014).
Methods: We employed an extensive expression analysis of all known Nox members and its adaptors at RNA and protein level in normal human fibroblasts and dermal fibroblasts from SSc patients by endpoint RT-PCR, Western blotting and immunofluorescence analysis using lased-scanning confocal microscopy. The impact of transforming growth factor-beta1 (TGF-beta1) on fibroblast activation was determined by real-time RT-PCR for collagen type I, fibronectin and alpha-smooth muscle actin, by procollagen type I C-terminal peptide ELISA and by immunofluorescence analysis. NADPH activity in membrane fractions was measured by enzyme assays. The mechanism of Nox4 induction by TGF-beta1 was investigated by in silico promoter analysis, siRNA of SMAD3 and pharmacological inhibition of SMAD3. Nox4 expression was blocked by Nox4 siRNA treatment, genetic ablation of Nox4 in murine fibroblasts, and pharmacological inhibiton of NADPH activity using diphenyleneiodonium (DPI). The role of Nox4 as a mediator of fibrosis was determined in the scleroderma bleomycin mouse model.
Results: Nox4 but not Nox1 or Nox2 was found to be expressed in both normal dermal fibroblasts and dermal fibroblasts from SSc patients. Within these cells Nox4 could be specifically located to the endoplasmic reticulum. The profibrotic cytokine transforming growth factor-beta1 (TGF-beta1) dose- and time-dependently increased Nox4 expression and enhanced NADPH enzyme activity. This effect of TGF-beta1 was SMAD3-dependent as shown by gene known-down and pharmacological inhibition of SMAD3. Nox4 did not only mediate TGF-beta1-induced collagen type I expression and secretion but regulated expression of the myofibroblast markers fibronectin and alpha-smooth muscle actin as collectively demonstrated by (1) pharmacological inhibition of NADPH activity with diphenyleneiodonium (DPI), (2) siRNA-mediated gene knock-down, and (3) genetic ablation of Nox4 employing fibroblasts form Nox4-/- mice. Importantly, administration of DPI *in vivo* reduced collagen expression, myofibroblast activation and skin fibrosis in the

bleomycin scleroderma mouse model. *In vivo* treatment of mice with Nox4 siRNA signficantly reduced bleomycin-induced skin fibrosis.
Conclusions: These findings strongly suggest that Nox4 is a promising candidate for clinical trials on SSc patients using Nox-subtype-specific NADPH inhibitors.

Parallel session 10 | Outcome measures

CO-41 | PREDICTION OF IMPROVEMENT IN SKIN FIBROSIS IN DIFFUSE CUTANEOUS SYSTEMIC SCLEROSIS - A EUSTAR ANALYSIS

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Introduction: The course of skin fibrosis varies widely across patients with diffuse cutaneous systemic sclerosis (dcSSc). An overall tendency towards improvement has been observed, but its determining factors are still unknown. Recognizing those patients prone to improve could avoid unnecessary use of therapy. Moreover, excluding "spontaneous improvers" could support cohort enrichment in clinical trials in skin fibrosis. In this study, we aim to identify predictors for improvement of skin fibrosis over 1 year in patients with dcSSc.
Methods: For this longitudinal analysis on the EUSTAR registry, we included patients with dcSSc, fulfillment of ACR1980 criteria, baseline modified Rodnan skin score (mRSS) >= 7, reported mRSS at 12 ± 2 months. Skin improvement was defined as decrease in mRSS of >5 points AND >= 25% within 1 year. Patients with an increase in mRSS at 1 year in the same amount were considered progressors. Candidate predictors of skin improvement were selected by expert opinion through the nominal group technique. Single and multiple imputation for missing data, predictive analysis by multiple logistic regression and validation of the final model using bootstrap were applied.
Results: 919 patients were analyzed; 218/919 (24%) patients had skin improvement and 95/919 (10%) had skin progression after 1 year. Interestingly, patients with high baseline mRSS were most likely to improve, whereas those with lower skin scores mostly progressed. The final predictive model from the multiple imputed dataset revealed high baseline mRSS as the strongest predictor for skin improvement at 1 year (OR 1.19, 95% CI [1.12-1.27], p<0.001), whereas presence of tendon friction rubs was inversely correlated (OR 0.61, 95% CI [0.40-0.93], p = 0.02). Alternative predictive modelling strategies (backward step-down selection, test on the available data), also revealed Anti-Scl70 negativity and normal erythrocyte sedimentation rate as significant predictors of skin improvement. High baseline mRSS remained the strongest predictor of skin improvement at 1 year in all exploratory models.
Conclusions: According to these evidence-based data, dcSSc patients with high baseline mRSS and absence of tendon friction rubs are most likely to experience improvement of skin fibrosis within 1 year, under standard of care. Therefore, they should be considered for exclusion from trial recruitment and perhaps also not selected for treatment (at least as long as the drug of choice has been shown to induce regression). Conversely, focus for treatment intervention and recruitment in clinical trials in skin fibrosis should shift to at risk patients with low to moderate skin fibrosis.

CO-42 | DEVELOPMENT OF A DISEASE DAMAGE INDEX IN SYSTEMIC SCLEROSIS USING CONSENSUS AND DATA DRIVEN METHODS

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Introduction: As there is currently no index for quantifying organ damage in systemic sclerosis (SSc), we sought to develop a disease damage index in SSc (SSc-DI) reflective of mortality and morbidity for use in interventional and observational studies.
Methods: The SSc-DI working group, together with patient partners and expert advisors from disciplines outside rheumatology, has developed a provisional SSc-DI mortality (SSc-DImort) and SSc-DI morbidity (SSc-DImorb) using the following steps: (1) Definition of the concept of damage in SSc using survey consensus; (2) Item generation through systematic literature review; (3) Item reduction using an online survey distributed to over 300 SSc experts internationally, together with Rasch modelling; (4) Two separate analyses using prospectively acquired data from the Australian Scleroderma Cohort Study (ASCS): (i) time-varying univariable cox proportional hazards regression survival analyses to determine the relationship between the reduced list of items and mortality, and (ii) random effects generalised linear panel methods to determine the univariable relationship between the items and morbidity as measured by the Short Form 36. Items were retained in both models if they showed a significant relationship (p<0.05). Multivariable regression was then performed to weight the item scores using the hazard ratio and coefficient calculations respectively.
Results: A total of 83 items from 7 domains were included in the online survey. A total of 93 responses from SSc experts were analysed. 63/83 items were retained based on survey responses (>60% experts deemed item appropriate). A further 27 items were dropped after Rasch modelling, leaving 31 items to be considered for inclusion in the SSc-DI. Eight of the 31 remaining items were not collected in the ASCS and therefore were unable to be analysed in regression models. The cohort data set consisted of 1,094 patients, 3,534 observations and 108 deaths (9.9%). The univariable relationship with death for the SSc-DImort and SSc-DImorb was statistically significant in 11 and 13 items respectively. Some items (3 in SSc-DImort and 6 in DImorb), although dropped in the Rasch analysis, were re-included in the final index by the investigators due to their strong associations with the end points.
Conclusions: The combined use of consensus and data driven methods has enabled development of a preliminary weighted Damage Index reflective of mortality and morbidity. By the time of the World SSc Congress 2016, further consensus will have been achieved at a face-to-face working group meeting at ACR ASM in San Francisco.

CO-43 | EUSTAR TASK FORCE FOR THE DEVELOPMENT OF A REVISED ACTIVITY CRITERIA FOR SYSTEMIC SCLEROSIS

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Introduction: Systemic sclerosis (SSc) is a polymorphic disease and defining disease activity in SSc cannot be done using a single variable. In 2001, the European Scleroderma Study Group (EScSG) identified 11 disease activity variables and developed a preliminary activity index. Its criterion validity is supported by several studies although some limitations were raised including a questionable face validity of hypocomplementemia. Here, we present the results of a EUSTAR study devoted to the optimization of a validated SSc activity index.
Our objective was to identify a validated set of activity criteria.

Methods: Investigators assigned an activity score on a 0-10 Lickert scale for 97 charts (randomly assigned, 3 investigators for each chart) retrieved from EUSTAR centres. The median score served as the gold standard. Two other investigators defined the disease status as inactive, moderately active, active or very active. Univariate and multivariate linear regression analyses were used to assess the performance of sets of clinical variables in predicting the "gold standard". Receiver operating curves assessed the efficiency of the index in separating active-very active from inactive-moderately active disease. The resulting index was validated on a second set of 60 charts by three different investigators on a VAS scale and defined as inactive, moderately active, active, and very active by other 2 investigators, blinded to the index.
Results: A weighted 10-point activity index was identified: Delta-skin, and Delta-heart-lung (patient-assessed worsening during the previous month), modified Rodnan skin score >18; the presence of digital ulcers; tendon friction rubs; erythrocyte sedimentation rate >50 mm/h; C reactive protein >1 mg/dl; diffusing capacity of the lung for CO <70% of predicted.
The accuracy of the 2015 index was shown using ROC curves. In the derivation sample of 97 patients, a value >= 1.75 had a sensitivity of 80.0% and a specificity of 89.4% for depicting disease activity, while using the 2001 EScSG activity index, an index >= 3 identified active-very active disease with a sensitivity of 57.5% and a specificity of 85.9%. The high performances of the 2015 index were further confirmed in an independent set of 60 additional Eustar patients.
Conclusions: A revised SSc activity index has been developed that performs better than the previous activity index.

CO-44 | COMPLEMENTARY VALUE OF ELF TEST AND NT-PROBNP IN REFLECTING FIBROSIS AND VASCULOPATHY IN SYSTEMIC SCLEROSIS

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Introduction: The ELF test and its components (PIIINP, TIMP-1 and HA) have been shown to correlate with skin, lung and overall fibrosis and not with any vascular manifestation of Systemic Sclerosis (SSc). On the contrary, NT-proBNP has been suggested to be useful for stratification of SSc patients, especially to identify those at risk of pulmonary hypertension. Aims of this study were: a) to validate ELF score and its single analytes on an independent cohort of scleroderma patients; b) to evaluate whether NT-proBNP could provide additional value to the development of a SSc-specific algorithm.
Methods: 250 sera from SSc patients from a single UK centre were analysed employing a high-throughput *in vitro* diagnostic of a routine NHS pathology lab to measure ELF score, its analytes and NT-proBNP levels. All patients fulfilled 2013 ACR/EULAR classification criteria for SSc. Clinical, laboratory and instrumental data were collected at time of sampling. Statistical analysis was performed using SPSS. P<0.05 was considered statistically significant.
Results: Multivariate analysis of ELF score (including the variables found statistically significant in univariate analysis) identified age, mRSS and DLCO% as independently associated with ELF score (p<0.0001 for all), confirming results previously published on an independent Italian cohort. As previously shown, ELF score and single analytes were not associated with heart and vascular manifestations of the disease. On the contrary NT-proBNP significantly correlated with severity of heart (p<0.0001) and of peripheral vasculopathy (p = 0.005). NT-proBNP levels were higher in patients with current digital ulcers (p = 0.001), digital pitting scars (p = 0.01), telangiectasias (p = 0.01), systemic arterial hypertension (p = 0.004), pulmonary artery hypertension (PAH) (p = 0.001), diastolic dysfuction (p = 0.002), reduced ejection fraction (p = 0.0002), arrhythmias (p<0.0001) and dyspnoea (p = 0.003) compared to those without the manifestation. Multivariate analysis identified presence of arrhythmias (p<0.0001), age (p<0.0001), PAH (p<0.001) and DLCO% (p = 0.006) as independently associated with NT-proBNP. All the biomarkers significantly correlated with total Medsger's severity scale and EScSG-activity index (p<0.0001).
Conclusions: Our findings validate the value of ELF score in a second independent cohort of 250 SSc sera and suggest that NT-proBNP has a complementary value correlating with other aspects of the disease such as PAH and heart severity. Longitudinal multicentres studies are warranted to determine the sensitivity to change and the predictive value of these biomarkers in SSc patients and to build up a new combined scleroderma specific algorithm including markers of fibrosis and vasculopathy.

CO-45 | THE SCLERODERMA FIBROTIC SCORE: A USEFUL SERUM TEST IN THE DIAGNOSIS OF EARLY SCLERODERMA

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Introduction: VEDOSS (Very Early Diagnosis of Systemic Sclerosis) is an international study aimed to identify, in patients at risk of SSc, factors predictive of progression and internal organ involvement. Recently, we have demonstrated that ELF serum test and its components (PIIINP, TIMP-1 and HA) correlate with severity of skin and lung fibrosis in SSc. Nevertheless its value in aiding early diagnosis of disease has still not been investigated. Our aim was to analyse the concentrations of ELF components and determine their potential diagnostic value in the VEDOSS patients.
Methods: Biosamples from 114 VEDOSS and 67 definite SSc fulfiling ACR/EUSTAR SSc 2013 classification criteria (33 diffuse; 34 limited SSc) were obtained from 2 centres. Serum concentrations of ELF components were determined on Siemens Advia Centaur platform. Logistic regression analysis of the results was performed employing classification of SSc as state variable.
Results: Among the 114 patients enrolled, 30 had primary Raynaud's phenomenon (PRP); 54 were classified as VEDOSS (RP, Puffy fingers, ANA, SSc specific autoantibodies or SSc-specific capillaroscopic pattern) not fulfilling 2013 ACR/EULAR criteria (score <9), while other 30 VEDOSS patients fulfilled new ACR/EULAR criteria despite lack of any sign of skin and internal organ involvement. ELF and its components correlated with age (p<0.05 for all). Logistic regression analysis using ELF variables identified a specific algorithm (SSc score) ranging from -3.92 to 5.77. The score showed good ability to discriminate between patients with definite SSc and VEDOSS patients already classified as SSc when compared to VEDOSS patients not yet classified as SSc (AOUC under ROC curve: 0,853 (95% CI 0,797-0.910)). Within the VEDOSS database, patients fulfilling SSc classification criteria, had average score of 0.44 vs -0.86 of patients not fulfilling the criteria, even after correcting for age (p = 0,026). Furthermore SSc score showed a fair ability to discriminate between the two groups (AOUC = 0.756, 95% CI 0.646-0.865). Moreover, definite SSc patients had significantly higher SSc score compared to VEDOSS patients classified as SSc, confirmed after age correction (p<0.000). No difference in SSc score has been observed between PRP and VEDOSS patients not yet classified as SSc.
Conclusions: Our data indicate that the SSc score is a simple serum test that can be used in patients with RP to aid in the early diagnosis of Scleroderma. Furthermore, the identification of VEDOSS patients with high SSc score test even in the absence of internal organ involvement can be used in intervention trials aimed to prevent further disease progression.

CO-46 | VERY EARLY AND EARLY SYSTEMIC SCLEROSIS PATIENTS IN THE SPANISH SCLERODERMA REGISTRY (RESCLE) COHORT

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Introduction: Systemic sclerosis is a complex disease difficult to diagnose at subclinical stages. The old 1980 American College of Rheumatology

criteria didn´t consider early disease. LeRoy and Medsger proposed a new classification in 2001 that included these patients, named limited SSc or pre-scleroderma. In recent years, using sensitive methods, we can identify subclinical organ involvement. According to this, we can now classify those patients at early stages into two subsets: Very Early SSc (presence of specific SSc autoantibodies and/or nailfold capillaries with scleroderma pattern) and Early SSc or pre-SSc (very early SSc characteristics plus at least one of the following: reduced lower esophageal sphincter pressure, reduced diffusing lung capacity for carbon monoxide or diastolic abnormalities at B-mode echocardiography, and/or digital ulcers, pitting scars, telangiectasias, calcinosis or arthritis.

Methods: The purpose of this study is to analyze the characteristics of patients with pre-scleroderma included in the Spanish Systemic Sclerosis Registry reclassified according the 2013 ACR/EULAR clasiffication criteria and the existence of organ involvement into pre-SSc or early SSc, comparing both groups and trying to identify risk factors of progression to definite SSc.

Results: 1,632 patients were included by March 2015, 36 of the very early subset and 111 of the early subset. Both groups were similar in terms of age at disease onset, Raynaud phenomenon (RP) duration or ANA positivity. The most frequent autoantibody specificity was anticentromere. The predominant capillaroscopic abnormalities showed a slow scleroderma pattern in both groups. Only one out of six esophageal manometries performed in the first group was altered, although not showing scleroderma involvement. Forty-one percent of the early SSc patients showed esophageal involvement. There were no statistically differences in both groups regarding to lung involvement. Diastolic abnormalities were detected in one pre-SSc patient (related to arterial hypertension) and in 11 of the early SSc patients. The median follow-up period was 2.24 years in the first group and 4.6 years in the second one. During follow up, only 2 pre-SSc patients met the 2013 ACR/EULAR criteria (limited cutaneous systemic sclerosis), unlike 20 (24%) of early SSc patients (4 early SSc, 2 diffuse cutaneous SSc and 14 limited cutaneous SSc) (p = 0.02).

Conclusions: Early SSc can evolve into definite SSc within a short-term follow up more frequently than very early SSc. A thorough internal organ evaluation is mandatory in patients with RP and positive ANA, nailfold abnormalities or both to identify patients at risk for developing systemic sclerosis over time.

CO-47 | FROM VEDOSS TO ESTABLISHED SYSTEMIC SCLEROSIS DIAGNOSIS ACCORDING TO THE NEW ACR/EULAR 2013 CLASSIFICATION CRITERIA: A CAPILLAROSCOPIC SURVEY

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Introduction: Nailfold Capillaroscopy (NC) is a diagnostic and sensitive tool in order to investigate Raynaud's phenomenon (RF) that is the hallmark of both Systemic Sclerosis (SSc) and Very Early Diagnosis Of ScS (VEDOSS) patients. Thus NC is largely used in patients with these conditions and NC specific abnormalities have become a minor criteria in ACR/EULAR SSc Criteria in 2013.

Methods: Aim of our study was to monitor NC changes in a group of 40 VEDOSS patients, selecting those who developed SSc at the follow-up. Moreover, we compared the NC features of these patients with NC of those VEDOSS patients who did not move to SSc, in order to find out NC features, if any, suitable as predictive risk factor for SSc at the time of VEDOSS diagnosis.

Results: In our 40 VEDOSS patients, 11 of them (all females, mean age 51 yrs) shifted to SSc in a follow-up time of 37 months (range 13-60 months).

When we compared basal NC of former VEDOSS developing SSc with 11 VEDOSS patients who did not develop ScS (all females, mean age 45 yrs) we found a significantly higher NC score (>1) in 9 versus 3 cases (p<0.03) and a larger mean apex width, 153 µm (range 43-393) versus 101 µm (range 26-233) (p<0.005) respectively.

Conclusions: Our study shows a progression of NC changes during the evolution of early SSc. Moreover we identified some NC features, such as higher NC score and larger apex width, whose presence at the very early diagnosis of SSc may suggests its development in an established disease, thus underlying the relevance of specific NC abnormalities as predictive risk factor.

Session 11 | Emerging therapies

CO-48| EFFICACY OF MYCOPHENOLATE MOFETIL (MMF) VERSUS ORAL CYCLOPHOSPHAMIDE (CYC) ON SKIN THICKNESS IN THE SCLERODERMA LUNG STUDY II

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Introduction: In a randomized controlled trial of mycophenolate mofetil (MMF) vs. oral cyclophosphamide (CYC) in patients with symptomatic scleroderma-related interstitial lung disease (SLS II), we assessed the impact of MMF and CYC on the modified Rodnan skin score (mRSS).

Methods: At 14 clinical centers throughout the United States, we enrolled 142 patients with SSc and ILD. Patients received mycophenolate (<= 3 g daily in divided doses) for two years or oral cyclophosphamide (<= 2 mg per kilogram of body weight every morning and placebo every evening) for one year followed by placebo twice daily for an additional year. mRSS was assessed every 6 months.

Results: The mean ± SD age was 52.3 ± 9.7 years and disease duration was 2.6 ± 1.8 years. The baseline mRSS was 14.0 ± 10.6 for CYC and 15.3 ± 10.4 for MMF groups; 58.5% were classified as diffuse cutaneous SSc and 42.5 were classified as limited cutaneous SSc. Both treatments were associated with improvement in mRSS over the period of 24 months. The mean improvement was numerically greater in limited cutaneous SSc in MMF vs. CYC and numerically greater improvement was seen with CYC vs. MMF in diffuse cutaneous SSc. In the diffuse cutaneous SSc, both treatments were associated with improvements that are greater than minimal clinically important differences at 12 and 24 months; 78% and 64%, in CYC and MMF groups improved by >= 5 units at 24 month.

Conclusions: In the SLS II, 2 year of daily MMF and 1-year of CYC was associated with clinically important improvements in mRSS in the diffuse cutaneous subset at 24 months. These data support use of MMF or CYC for management of skin fibrosis in early diffuse cutaneous SSc.

CO-49 | BASELINE CHARACTERISTICS AND TREATMENT CHOICES IN PATIENTS WITH EARLY DIFFUSE CUTANEOUS SYSTEMIC SCLEROSIS (ESOS COHORT)

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Introduction: ESOS (European Scleroderma Observational Study) is a prospective observational study to compare effectiveness of different immunosuppressants currently used by clinicians treating early diffuse cutaneous systemic sclerosis (dcSSc). Here we report baseline characteristics of the study cohort and compare these between the 4 different treatment protocols (methotrexate, mycophenolate mofetil, cyclophosphamide, and 'no immunosuppressant').

Methods: Target recruitment was achieved, with 326 patients from 50 centres. The baseline characteristics considered in this analysis were: age, gender, antibody status, duration of skin thickening, modified Rodnan skin score (mRSS), internal organ involvement, indices of functional ability/general health (Health Assessment Questionnaire Disability Index [HAQ-DI], SF-36), hand function (Cochin), fatigue (FACIT), previous/current use of steroids and previous use of immunosuppressants.

In an observational study, it is possible that the identified differences in outcome between the treatment protocols are due to differing patient characteristics between the treatment groups, rather than different effects of the treatments themselves (confounding by indication). To identify characteristics that vary across protocols, Fisher's exact test (for categorical variables) or Kruskal-Wallis' test (for continuous variables) is applied. Additional post-hoc tests were performed to single out the differences of the characteristics in one protocol compared with each other.

Results: At baseline there were differences between groups in gender, duration of skin thickening, pulmonary fibrosis, renal, cardiac and muscle involvement, Cochin hand function score, steroid and immunosuppressant use. Differences in age, antibody status, skin score, pulmonary hypertension, gastrointestinal involvement, HAQ-DI and SF-36 scores between protocols were not statistically significant.
At baseline, patients in the cyclophosphamide protocol had higher rates of cardiac involvement and pulmonary fibrosis, and poorer hand function, compared to at least one of the other protocols. They were more likely to have already taken immunosuppressant or steroids prior to entering the study and men were over-represented. Patients assigned to methotrexate had a higher incidence of muscle involvement. Patients enrolled in the "no immunosuppressant" arm had longer duration of skin thickening than patients on other protocols.
Conclusions: The short disease duration of the ESOS cohort suggests that patients with early disease (and therefore likely to progress) have been enrolled. Baseline characteristics were analysed and several potential confounders were identified: these will be taken into account in the longitudinal analysis examining treatment response. Our findings underscore the high disease burden of patients with early dcSSc in terms of internal organ involvement and functional impact.

CO-50 | INHIBITION OF MYELOID-ASSOCIATED GENE EXPRESSION IN SKIN BIOPSY SAMPLES OF SYSTEMIC SCLEROSIS PATIENTS TREATED WITH TOCILIZUMAB

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Introduction: Systemic sclerosis (SSc) is a progressive, debilitating disease with limited treatment options. IL-6 has been implicated in disease pathogenesis. Tocilizumab (TCZ), an IL-6Rα inhibitor, was evaluated in the 2-year faSScinate study, a randomized, double-blind, placebo-controlled trial. At week 24 (primary end point), favorable trends in skin score for TCZ were detected though the primary skin score end point was not met. In addition, smaller declines in FVC were observed in the TCZ-treated patients.
Methods: Eighty-seven patients with active SSc were randomly assigned 1:1 to subcutaneous TCZ 162 mg or placebo (PBO) weekly for 48 weeks. The primary end point was mean change in modified Rodnan skin score from baseline at week 24. Gene expression analysis was performed on skin biopsy samples collected at baseline and week 24. First, genomewide expression analysis was conducted on all available biopsy samples and on biopsy samples of age- and sex-matched healthy volunteers (HVs) using custom Agilent 60-mer microarray (Epistem, Manchester, UK). Based on these data, 86 genes representing fibrosis, IFN, and myeloid pathways were selected for more quantitative gene expression analysis using nCounter technology (NanoString Technologies, Seattle, WA, USA). CCL18 serum levels were determined using an IMPACT-based immunoassay (Roche Diagnostics, Penzberg, Germany).
Results: Of the 86 genes selected for follow-up expression analysis, 75 were, on average, significantly overexpressed in SSc patients compared with HVs. Analysis of genes significantly downregulated after TCZ treatment and stable or increased with PBO identified a subset of 14 genes highly enriched for myeloid-associated genes, including genes associated with M2 macrophages. All 14 genes were overexpressed in SSc patients compared with HVs. No effect of TCZ on the fibrosis and IFN pathways was detected. Serum levels of the M2 macrophage chemokine CCL18 revealed a rapid and sustained decrease from elevated levels in the TCZ group to close to levels in healthy controls but not in PBO controls.
Conclusions: The effect of TCZ on myeloid-associated genes may reflect inhibition and/or depletion of skin-infiltrating macrophages. In addition, the effect of TCZ on CCL18 (RNA and protein), CD163, MS4A4A, and MSR1 suggests a specific inhibitory effect of TCZ on M2 macrophages, which are known to promote fibrosis and inflammation. These findings represent a potential novel mode of action for TCZ in SSc, which will be further investigated in the upcoming phase 3 study of TCZ in SSc.

CO-51 | TREATING SCLERODERMA OF THE FACE AND HANDS WITH FAT AND STROMAL VASCULAR FRACTION

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Introduction: Since 2009, we have treated with fat and StRomal Vascular Fraction systemic sclerosis patients.
Methods: We treated the faces of 14 patients using micro-injection with a minimally invasive closed filtration system, aiming at volumetric and trophic effects. We used 16 to 22 cc of fat, which was harvested with 14 gauge or 2 mm cannulae, and reinjected with 21 gauge or 0.8 mm cannulae.
In addition, we treated 12 patients (24 hands) with the StRomal Vascular Fraction, aimed at an angiogenic and anti-fibrotic effects. We harvested 135-270 g of fat which allowed us to get 5 cc of stRomal vascular fraction with the Celution system. We got on average 50 x 106 cells which were divided into 10 doses of 1 cc. A subcutaneous injection was performed in the patient's every finger with 25 gauge or 0.5 mm cannulae.
Both facial and finger procedures were performed under local anaesthesia.
Results: On the face, we observed a continuous improvement process. The pain was reduced in the tempoRomandibular joints, the tissues softened, the buccal aperture was improved with special consideration to the aesthetic enhancement. The improvement was immediately assessed. Some patients underwent a second injection procedure, 2 years after the first one.
With respect to the hands, we observed spectacular results, with a very rapid improvement of the vascularisation of the fingers and later of trophic disorders that allowed a functional enhancement and a better quality of life.
Results persist beyond the second year. No complications were observed.
Conclusions: We conclude that microfat grafting on the face is efficient to treat functional and aesthetic disorders. The injection of stRomal vascular fraction in fingers triggers an obvious functional improvement in everyday life activities. Two randomized clinical trials are underway in France and USA. Overall, these safe and minimally invasive techniques provide an important benefit in terms of aesthetic and functional improvements.