setting, our results confirm that defSSc might represent an intermediate entity between pre-clinical stages and the most severe subsets of disease, thereby opening new perspectives on SSc pathophysiology and disease interception.

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AB0178 PHENOTYPING OF NATURAL KILLER (NK) RECEPTORS ON NK AND NKT-LIKE CELLS DISCLOSES DEFECTIVE IMMUNE-REGULATORY CAPABILITY IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by dysregulation of the immune system, vasculopathy and fibrosis of the skin and internal organs. Natural Killer (CD56⁺CD3⁻, NK) and NKT-like (CD56⁺CD3⁺) cells display receptors (NKR) whose expression pattern determines their cytotoxic and immune-regulatory activity. The role of NK and NKT-like cells in the dysregulation of the immune system in SSc has not been fully elucidated yet.

Objectives: To improve our knowledge on the contribution of NK, NKT-like and a subset of NKT cells expressing invariant TCR (iNKT) in SSc development, we performed a broad phenotyping of NKR in the circulation of SSc patients, including subjects with pre-clinical SSc.

Methods: NKR were assessed by flow cytometry using two 13-color panels on whole blood of 84 SSc patients and 20 healthy controls (HC). In particular, 15 patients with early SSc (EaSSc) without signs or symptoms of evolutive disease (2001 LeRoy and Medsger criteria¹), 24 patients with definite SSc without skin or lung fibrosis (defSSc), 26 patients with limited (lcSSc) and 19 patients with diffuse cutaneous SSc (dcSSc) (2013 ACR/EULAR classification criteria for SSc²) were included. NK degranulation in response to K562 target cells was assessed in lcSSc and dcSSc patients versus HC.

Results: The number of circulating lymphocytes, NKT-like and iNKT cells - but neither CD3⁺ T nor NK cells - was reduced in dcSSc versus HC. NKp46⁺ NK cells co-expressing NKG2D and CD16 were decreased in dcSSc versus HC and EaSSc. Consistently with these observations, dcSSc exhibited lower degranulation capability. (CD57⁺)KIR⁺ and activating NKR-expressing NKT-like cells were diminished in both dcSSc and lcSSc versus HC.

Conclusions: dcSSc patients showed a defective NK cytotoxicity potential, possibly due to the decreased NKp46⁺ fraction. The regulatory, cytolytic KIR⁺ NKT-like fraction was also reduced with a parallel decrease of activating receptors expression in both lcSSc and dcSSc. Overall these results point towards an impairment of NK and NKT-like cells as immune check-points in fibrotic SSc.

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AB0179 DEGRADATION OF TYPE VII COLLAGEN (C7M) IS ASSOCIATED WITH SYSTEMIC SCLEROSIS – DEVELOPMENT OF A NOVEL NEO-EPI-TOPE SPECIFIC ASSAY

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Background: Type VII collagen (col7) is the main component of the anchoring

fibrils that connects the basement membrane to the underlying interstitial matrix and has mainly been investigated for its role in blistering skin diseases. It has been investigated for its role in dystrophic epidermolysis bullosa, a severe skin disease. Furthermore, increased levels of type VII collagen in skin has been reported for patients with systemic sclerosis (SSc).

Objectives: The objective was to develop and characterize a blood-based marker assessing col7 degradation in patients with SSc.

Methods: We identified a specific fragment of col7 in serum from COPD patients, which was not found in controls, using mass spectrometry. A monoclonal antibody was raised against the first ten amino acids of the neo-epitope (KLH-CGG-GPPGPPGRLV) and employed in a competitive ELISA (C7M). The C7M assay was validated technically and was subsequently evaluated in 2 cohorts including SSc patients. The first cohort (SSc#1; n=35) consisted of early (<2 years of SSc symptoms; n=16) and late (>10 years of disease with stable skin for at least 6 months, n=19) diffuse SSc patients, while the second cohort (SSc#2; n=119) consisted of limited (n=78) and diffuse (n=41) SSc patients. Serum C7M levels were likewise measured in healthy subjects and compared to the levels of SSc patients using the Kruskal-Wallis test with Dunn's multiple comparisons test comparing healthy individuals with the two SSc cohorts.

Results: A technically robust competitive ELISA (C7M), which was highly specific for a col7 fragment was developed. The assay showed acceptable inter- (13%) and intra-assay (9%) variation, linearity (102% dilution recovery), analyte stability (102% recovery after 4 freeze/thaw cycles), and interference.

The C7M marker was evaluated by comparing serum levels in healthy donors with patients with SSc (Figure). Serum C7M levels were not associated with age, gender, BMI, or disease duration. The geometric mean serum C7M level in healthy donors was 4.6 ng/mL [95% CI 3.7–5.6 ng/mL]. The geometric mean serum C7M levels were significantly elevated in both cohorts of patients with SSc (SSc#1, 13.6 ng/mL [95% CI 11.1–16.5], p<0.0001; SSc#2, 9.2 ng/mL [95% CI 8.3–10.2], p<0.0001). Furthermore, a significant difference were observed between the two cohorts (P=0.05).

Conclusions: The C7M ELISA enabled quantification of type VII collagen degradation in serum. Elevated serum C7M levels indicated that the remodeling of type VII collagen was significantly increased in patients with SSc, suggesting a pathological role.

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AB0180 BIOMARKERS OF EXTRACELLULAR MATRIX REMODELING ARE ASSOCIATED WITH ACUTE EXACERBATIONS OF IDIOPATHIC INTERSTITIAL PNEUMONIA

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Background: Idiopathic interstitial pneumonia (IIP) is characterized by an increased rate of extracellular matrix (ECM) turnover resulting in fibrosis. The pathogenic remodeling includes increased levels of protein synthesis and degradation mediated by proteases such as matrix metalloproteinases (MMPs). Acute exacerbations of IIP (AE-IIP) represent periods of increased disease activity. Pulmonary involvement, especially pulmonary fibrosis, is common in patients suffering from systemic sclerosis (SSc) and ankylosing spondylitis (AS).

Objectives: The objective was to investigate if ECM remodeling was altered during AE-IIP by serological neo-epitope biomarkers.

Methods: Serum samples were collected from patients with IIP at clinically stable disease (S-IIP, n=29) and at AE-IIP (n=68). Of these, 11 and 28 patients, respectively, had idiopathic pulmonary fibrosis (IPF). 28 IIP patients had paired samples. Biomarkers released from MMP-mediated degradation of collagen type I (C1M), III (C3M), IV (C4M), and VI (C6M), elastin (ELM7), versican (VCANM), biglycan (BGM), and C-reactive protein (CRPM) were assessed in serum by competitive ELISAs utilizing neo-epitope specific monoclonal antibodies. Data were analysed using Mann-Whitney test, Wilcoxon test, Spearman's rank correlation, and Kaplan-Meier curves as appropriate.

Results: Mean age of patients was 71 (range 54–86) at AE-IIP and 69 (range 55–83) at S-IIP. Mean forced vital capacity in percentage of predicted value (%FVC) was 55.6% (SD 19.5) at AE-IIP and 79.0% (SD 26.5) at S-IIP. Serum

levels of C4M, C6M, and CRPM at AE-IIP were positively correlated with %FVC ($r=0.37$, $p=0.008$; $r=0.36$, $p=0.011$; $r=0.33$, $p=0.020$; respectively). Serum levels of C4M ($p=0.002$) and C6M ($p=0.024$) were increased while ELM7 ($p=0.024$) and VCANM ($p<0.0001$) were decreased at AE-IIP as compared with S-IIP when analyzing all patients. IPF patients had lower levels of VCANM at AE-IIP ($p=0.0001$). Patients with paired samples showed significantly elevated serum levels of C4M ($p=0.004$) and decreased levels of ELM7 and VCANM ($p=0.036$ and $p=0.0001$, respectively) at AE-IIP. C1M and C6M levels at AE-IIP were borderline related to mortality outcome for IPF patients (both $p=0.059$) with levels above median associated with a higher risk of mortality. Analyses of all or paired patients showed no associations with mortality.

Conclusions: Serological levels of neo-epitope biomarkers of ECM degradation were associated with AE-IIP and weakly with mortality outcome. These results indicate that the rate of ECM remodeling in the lungs of patients with IIP is significantly altered during periods of high disease activity such as an acute exacerbation. The difference in degradation profile for the proteins studied is intriguing and indicate activation of different processes contributing to AE-IIP. Neo-epitope biomarkers of the ECM might be useful in identifying patients diagnosed with rheumatic disease as SSc and AS with pulmonary involvement.

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AB0181 DIFFUSING CAPACITY AND CLINICAL CHARACTERISTICS OF PATIENTS WITH SYSTEMIC SCLEROSIS – DATA FROM THE GERMAN NETWORK FOR SYSTEMIC SCLEROSIS

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Background: Lung involvement, i.e. interstitial lung disease (ILD) and pulmonary hypertension (PH), is common in patients with systemic sclerosis (SSc), significantly limiting quality of life and survival. Data on clinical correlations between lung function and clinical subsets of SSc are sparse.

Objectives: To investigate the relationship of DLCO and clinical characteristics in patients SSc patients within the registry of the German Network for Systemic Scleroderma.

Methods: Clinical data of the patient registry, currently including DLCO data of 1917 patients were evaluated. In total, these patients were clinically evaluated 5997 times (i.e., at the first visit and during follow-up visits). At the initial visit and during follow-up DLCO levels were correlated with clinical characteristics.

Results: At initial presentation, 64% of the patients had DLCO levels <75% predicted. Impaired DLCO levels were observed in 74% of dcSSc patients, in 64% of SSc-Overlap patients and 57% of lcSSc patients ($p<0.0001$). Furthermore, male patients (62%), patients with PH (80%), ILD (80%), dyspnea (78%), and those with presence of anti-topoisomerase I antibodies (71%) exhibited significantly more often DLCO levels <75% ($p<0.01$). Patients suffering from dcSSc had the lowest DLCO levels (mean value, 62%), followed by patients with SSc-Overlap syndromes (mean value, 67%) and lcSSc patients (mean value, 70%) revealing significant differences between subsets. Long-term follow-up evaluation (mean follow up, 6.0 years) revealed that in comparison to lcSSc patients dcSSc patients (OR 2.1; $p<0.0001$; 95%-CI 1.7–2.5) and SSc-Overlap patients (OR, 1.55; $p<0.0001$; 95% CI, 1.2–2.0) had a significantly increased risk to a decrease in DLCO levels <75%.

Conclusions: Impairment of pulmonary function as determined by diffusing capacity DLCO is more common and more pronounced in patients with dcSSc and SSc-Overlap Syndrome compared to lcSSc. DLCO may be useful for diagnosing and monitoring pulmonary involvement in SSc.

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AB0182 IN VITRO CHARACTERIZATION OF DERMAL FIBROBLASTS FROM SYSTEMIC SCLEROSIS PATIENTS

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Background: Systemic sclerosis (SSc) is characterized by progressive fibrosis of the skin and/or internal organs. Skin fibrosis is mainly due to the excessive production of extracellular matrix (ECM) proteins from dermal activated fibroblasts. The high amounts of transforming growth factor-beta (TGFbeta) production induce fibroblast proliferation and their transition into profibrotic myofibroblasts. Consequently, the increased presence of alpha-smooth muscle actin (alpha-SMA)-positive myofibroblasts in affected tissues is associated with fibrotic tissue remodelling, that characterize SSc [1–4].

Objectives: To characterize dermal fibroblasts from SSc patients by evaluating the gene expression of specific phenotypic and profibrotic markers: fibrillar collagen type I (COL I), fibronectin (FN), fibroblast-specific protein 1 (S100A4), TGFbeta and alpha-SMA (marker of myofibroblasts).

Methods: Human dermal fibroblasts were obtained by skin biopsy from affected areas of 6 active lcSSc patients after written informed consent and approval of medical ethics committee. SSc patients fulfilled the new EULAR/ACR criteria for SSc and they were treated only with various vasodilators (no severe clinical SSc complications were present at the time of skin sampling). Human SSc dermal fibroblasts were cultured up to 80% of confluence and at the third to fifth subpassages were used for experiments. Total RNA was extracted and quantitative real-time PCR (qRT-PCR) was performed to evaluate the gene expression of relevant fibroblast markers: COL I, FN, S100A4, TGFbeta and alpha-SMA.

Results: In human SSc fibroblasts the gene expression of the relevant markers COL I, FN and S100A4 resulted of 10^7 RNA copies for each gene. Interestingly, in the same tested fibroblasts, the profibrotic alpha-SMA gene expression (marker of myofibroblasts) was similarly found highly expressed, with a result of 10^7 gene copies. On the contrary, TGFbeta showed a much lower gene expression (10^3 copies) compared to the other investigated genes.

Conclusions: In human SSc cultured dermal fibroblasts, the gene expression of ECM proteins (COL I and FN), was found associated with a relevant gene expression of S100A4 and alpha-SMA, confirming their myofibroblast phenotype, as highly differentiated. Interestingly, TGFbeta gene appears to be less expressed. It could be concluded that myofibroblasts from the SSc skin mainly undergo the effects of the tissutal TGFbeta, since it seems that they do not contribute to its further production once differentiated.

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AB0183 ISOLATION AND CHARACTERIZATION OF CIRCULATING FIBROCYTES FROM SYSTEMIC SCLEROSIS PATIENTS: AN VITRO INVESTIGATION

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Background: In physiological and pathological conditions, such as in tissue repair and in fibrosing diseases (i.e. systemic sclerosis, SSc), the importance of circulating fibrocytes relies on the capacity of such cells to migrate into scleroderma-affected tissues (through CXCR4/CXCL12 interaction) and to differentiate into fibroblasts/myofibroblasts, inducing fibrosis (through matrix protein deposition) (or both) [1–3]. In addition, fibrocytes seem to exert immunomodulatory effects, expressing class II major histocompatibility complex molecules (HLA-DP, -DQ, and -DR) [4].

Objectives: To isolate fibrocytes from Circulating Progenitor Cells (CPCs - peripheral blood mononuclear cells PBMCs) of SSc patients and healthy subjects and to identify them by fluorescence-activated cell sorter analysis (FACS), using their specific markers: the leukocyte common antigen CD45, collagen I (COL I), the chemokine receptor CXCR4 and HLA-DR [2].

Methods: Samples were collected, at basal time (t0), from 11 SSc patients, affected by SSc (treated only with different vasodilator drugs) and 5 healthy