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.

Acknowledgements: The Danish Research Foundation

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5699

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

P. Moinzadeh¹, N. Blank², E. Siegert³, J. Henes⁴, M. Worm⁵, C. Sunderkoetter⁶, M. Schmalzing⁷, A. Kreuter⁸, C. Gunther⁹, L. Susok¹⁰, G. Zeidler¹¹, I. Koetter¹², U. Mueller-Ladner¹³, T. Krieg¹, A. Juche¹⁴, T. Schmeiser¹⁵, G. Riemekasten¹⁶, E. Aberer¹⁷, N. Gaebele-Wissing¹⁸, J.H.W. Distler¹⁹, M. Sárdy²⁰, C. Pfeiffer²¹, K. Kuhr²², N. Hunzelmann¹, F. Bonella²³, M. Kreuter²⁴. ¹Dermatology, University Hospital Cologne, Cologne; ²Rheumatology, University Hospital Heidelberg, Heidelberg; ³Rheumatology, Charité Universitätsmedizin Berlin, Berlin; ⁴Rheumatology, University Hospital Tuebingen, Tuebingen; ⁵Dermatology, Charité Universitätsmedizin Berlin, Berlin; ⁶Dermatology, University Hospital Muenster, Muenster; ⁷Rheumatology, University Hospital Wuerzburg, Wuerzburg; ⁸Dermatology, HELIOS St. Elisabeth Klinik Oberhausen, Oberhausen; ⁹Dermatology, University Hospital Carl Gustav Carus, Dresden; ¹⁰Dermatology, St. Josef Hospital Bochum, Bochum; ¹¹Rheumatology, Johanniter-Krankenhaus im Fläming Treuenbrietzen, Treuenbrietzen; ¹²Rheumatology, Asklepios Klinik Altona, Hamburg; ¹³Rheumatology, Justus Liebig University Giessen, Kerckhoff Clinic, Bad Nauheim; ¹⁴Immanuel Krankenhaus Berlin-Buch, Berlin; ¹⁵Rheumatology, Krankenhaus St. Josef, Wuppertal; ¹⁶Rheumatology, University Medical Center-UKSH, Luebeck, Germany; ¹⁷Dermatology, Medical University of Graz, Graz, Austria; ¹⁸Dermatology, HELIOS University Hospital Wuppertal, Wuppertal; ¹⁹Rheumatology, University Hospital Erlangen, Erlangen; ²⁰Dermatology, Ludwig Maximilians University Hospital, Munich; ²¹Dermatology, University Medical Center Ulm, Ulm; ²²IMSIE, University of Cologne, Cologne; ²³Unit for interstitial lung diseases, Ruhrlandklinik University Hospital Essen, Essen; ²⁴Center for interstitial and rare lung diseases, Thoraxklinik, University Hospital Heidelberg, Heidelberg, Germany

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.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.3474

AB0182 IN VITRO CHARACTERIZATION OF DERMAL FIBROBLASTS FROM SYSTEMIC SCLEROSIS PATIENTS

R. Brizzolara¹, P. Montagna¹, S. Soldano¹, A.C. Trombetta¹, B. Ruaro¹, A. Sulli¹, S. Scabini², E. Stratta², V. Smith³, M. Cutolo⁴. ¹Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, IRCCS San Martino; ²Oncologic Surgery, Department of Surgery, IRCCS San Martino IST, Genoa, Italy; ³Department of Rheumatology, Ghent University Hospital, Ghent, Belgium; ⁴Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, Genoa, Italy

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.

References:

- [1] Cutolo M. et al. *J Rheumatol* 2015;42:3.
- [2] Brunasso A.M. et al. *F1000Research* 2016;5:723.
- [3] Hinz B et al. *Am J Pathol* 2012;180:1340–55.
- [4] Dumoitier N. et al. *Arthritis Rheumatol* 2016 [Epub ahead of print].

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.3687

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

R. Brizzolara¹, P. Montagna¹, S. Soldano¹, A.C. Trombetta¹, P. Contini², V. Smith³, B. Ruaro¹, A. Sulli¹, M. Cutolo¹. ¹Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, IRCCS San Martino; ²Division of Clinical Immunology, Department of Internal Medicine, University of Genoa, Genoa, Italy; ³Department of Rheumatology, Ghent University Hospital, Ghent University, Ghent, Belgium

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

subjects (CNTs). PBMCs, isolated from 9 among the SSc patients, were cultured on fibronectin-coated plates [5]. The non-adherent cells were removed and after 8 days (t8) of culture (standardized time), the adherent spindle shaped cells were lifted through incubation in 0.05% EDTA (ice-cold). Fibrocyte identification (at both t0, t8), was performed by FACS, using anti-CD45, anti-COL I, anti-CXCR4 and anti-HLA-DR monoclonal antibodies.

Results: FACS analysis revealed that, at basal time (t0), among the CD45+ cells, the percentage of fibrocytes, identified as triple positive (CD45+, COL I+, CXCR4+) was 1.0±1.2% in SSc patients and 0.4±0.3% in healthy subjects (CNTs). In addition, the HLA-DR expression on fibrocytes in both SSc patients and CNTs showed low values (22.1±21.1% and 7.1±6.1%, respectively).

After 8 days (t8) of culture, fibrocytes presented adherent and spindle shaped morphology. Interestingly, the FACS analysis at t8 of culture, demonstrated that the percentage of SSc fibrocytes CD45+, COL I+, CXCR4+ increased up to 52.8±27.1%, compared to basal time (t0), as well as strongly increasing the HLA-DR+ expression (90.1±22.7%).

Conclusions: Fibrocytes isolated from CPCs of SSc patients were confirmed to express CD45, COL I and CXCR4 molecules, but in very low percentage at the beginning. Already after 8 days of culture in proper conditions, the percentage of differentiated fibrocytes (CD45+, COL I+ and CXCR4+) from SSc patients, increased up to 50 times and the HLA-DR expression increased up to 68%. Additional markers of progressive fibrocyte differentiation are now under test to further characterize the fibrocytes phenotype(s) of SSc patients vs healthy controls.

References:

- [1] Chesney J et al. Proc. Natl. Acad. Sci. 1997; 94:6307–6312.
- [2] Bucala R. Mol Med 2015;21:S3–S5.
- [3] Brunasso A.M. et al. F1000Research 2016;5:723.
- [4] Blakaj and Bucala. Fibrogenesis & Tissue Repair 2012;5:S6.
- [5] Pilling D et al. J Immunol Methods 2009; 351:62–70.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4963

AB0184 CHROMOSOMAL ABERRATIONS ARE ASSOCIATED WITH DISEASE SEVERITY IN A MOUSE MODEL OF BLEOMYCIN-INDUCED SCLERODERMA

R. Luisetto¹, M. Beggio², A. Scanu², F. Leite de Oliveira³, E. Pigatto², A. Peruffo⁴, E. Zanatta², F. Oliviero², P. Sfriso², L. Punzi², F. Cozzi². ¹Dept of Surgery, Oncology and Gastroenterology; ²Dept of Medicine- Rheumatology Unit, University of Padova, Padova, Italy; ³Institute of Biomedical Sciences, Federal University Rio de Janeiro, Rio de Janeiro, Brazil; ⁴Dept. of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy

Background: Scleroderma (SSc) is a connective tissue disease characterized by immunomediated inflammation and fibrosis. The presence of autoantibodies against chromosome associate proteins, such as anti-centromere proteins and anti-topoisomerase I, was observed in SSc patients, however genotoxic events have never been evaluated (2).

Objectives: To assess the presence of aneuploidia, micronuclei (MN) and other chromosomal aberrations in peripheral lymphocytes in a bleomycin (BLM)-induced scleroderma mouse model.

Methods: BALB/c mice (n=20 per condition) were injected subcutaneously daily with BLM 2mg/kg or PBS (control) for 14 days. Urine was collected weekly and proteinuria was measured. At day 15 mice were sacrificed and organs harvested for histological analysis. The MN assay was performed on cultured peripheral lymphocytes isolated from mice spleens. Cells were treated with cytochalasin B (10 µg/ml) for 24 h and a minimum of 1000 binucleated cells/mouse were scored following the criteria of Fenech (1). The antikinetochore (CREST) staining was used to determine the origin of MN observed.

Results: In BLM treated mice was observed huge perivascular lung fibrosis and significant skin involvement. 17 out of 20 mice developed acute renal involvement with mean proteinuria levels of 730±48 mg/dl. In comparison with the control mice, a significant increase in MN number was observed in BLM treated mice (57.8±4.4 vs 6.3±0.6, p<0.05). CREST staining was higher in MN derived from BLM treated mice (16.4±1.1 vs 3.7±0.7, p<0.025), indicating that in this group lymphocyte MN arose mainly from lagging chromosomes. In addition, an increased frequency of ring chromosome was observed in mice with greater skin fibrosis and renal involvement. A correlation between the presence of CREST stained MN and disease severity parameters as renal failure, lung and skin fibrosis was observed (respectively R=0.4095; R=0.7507 and R=0.9471).

Conclusions: This study provides experimental evidence that significant chromosomal aberrations seem to be related to the severity of disease in a mouse model of SSc. Further investigations on lymphocytes obtained from SSc patients could be matched with results observed in mouse model to confirm the relationship between severity of the disease and chromosomal abnormalities.

References:

- [1] Fenech M. Cytokinesis-block micronucleus cytome assay. Nature Protocols 2006, 2(5): 1084–1199.
- [2] Korman BD and Criswell LA. Recent advances in the genetics of systemic sclerosis: toward biological and clinical significance. Curr Rheumatol Rep 2015, 17(3): 21–34.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5043

AB0185 ENDOTHELIN-1 STIMULATES THE PROFIBROTIC ALTERNATIVELY ACTIVATED PHENOTYPE IN CULTURED HUMAN MACROPHAGES ISOLATED FROM SYSTEMIC SCLEROSIS PATIENTS

S. Soldano¹, P. Montagna¹, R. Brizzolara¹, A.C. Trombetta¹, C. Corallo², C. Pizzorni¹, S. Paolino¹, A. Sulli¹, M. Ghio¹, V. Smith³, N. Giordano², M. Cutolo¹. ¹Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, Genoa; ²Medicine, Surgery and Neurosciences, University of Siena, Siena, Italy; ³Department of Rheumatology, Ghent University Hospital, Ghent University, Ghent, Belgium

Background: In the damaged tissue of systemic sclerosis (SSc) patients, the presence of immune inflammatory infiltrate, characterized by T-cells and macrophages, represents an important early pathological event in the fibrotic process (1).

In the macrophage population, the alternatively activated (M2) macrophages were observed in both peripheral blood and damage tissues of SSc patients and they participate in the fibrotic process exerting profibrotic effects, primarily by the production and release of transforming growth factor β1 (TGFβ1) (1,2). These cells are characterized by the expression of specific phenotype markers, such as mannose receptor (CD206) and scavenger receptors (CD204 and CD163), and the production of specific chemokines (i.e. macrophage derived chemokine, CCL-22) (3). Endothelin-1 (ET1) plays an important role in the fibrotic process of SSc by inducing the profibrotic phenotype in endothelial cells and fibroblasts (4).

Objectives: To evaluate the ability of ET1 to upregulate the profibrotic gene expression profile characterizing the M2 macrophages in cultured monocytes/macrophages isolated from SSc patients.

Methods: Human monocytes were isolated from peripheral blood mononuclear cells of 4 SSc patients (mean age 59±11 years, 2 females and 2 males, treated with vasodilator drugs) using a monocyte isolation kit. Cultured cells were maintained in RPMI growth medium for 24hrs and then treated with ET1 (100nM) for 6 days or treated for 1hr with ET receptor antagonist (ET_{A/B}RA, bosentan 10µM) before stimulation with ET1. Cultured cells maintained in RPMI growth medium were used as untreated cells.

Gene and protein expression of M2 phenotype markers and TGFβ1 were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR) and Western blotting (WB). Statistical analysis was carried out using a Mann-Whitney non-parametric test.

Results: In cultured SSc macrophages, ET1 induced the upregulation of the gene expression of CD206, CD204, CD163, and CCL22 compared to untreated cells. Moreover, ET1 increased the TGFβ1 gene expression compared to untreated cells. ET_{A/B}RA partially contrasted the gene expression of CD204 and CD206. However, ET_{A/B}RA antagonized the ET1-mediated increase in the gene expression of CD163, CCL22 and TGFβ1. WB confirmed the results obtained by qRT-PCR.

Conclusions: Preliminary results showed the ability of ET1 to stimulate a profibrotic M2 phenotype in cultured human SSc macrophages characterized by the overexpression of TGFβ1, and this process is partially contrasted by the action of ET_{A/B}RA.

References:

- [1] Higashi-Kuwata N et al. Arthrit Res Ther. 2010;12:R128.
- [2] Stifano G et al. Curr Rheumatol Rep. 2016;18:2.
- [3] Wynn TA et al. Nat Rev. 2013;496:445–55.
- [4] Cipriani P et al. J Rheumatol. 2015;42:1808–16.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5857

AB0186 ASCA (ANTI-SACCHAROMYCES CEREVISIAE ANTIBODY) IN SCLERODERMA

A. Fedrigo, T.A.F.G. dos Santos, A. Bortoluzzi, T. Skare, R. Nishihara. Rheumatology Unit, Hospital Universitário Evangélico de Curitiba, Curitiba, Brazil

Background: The majority of Scleroderma (SSc) patients have gastrointestinal involvement. Motility is usually compromised but few studies address permeability changes in the intestinal wall. ASCA is an antibody directed against mannans in the cell wall of *Saccharomyces cerevisiae*, the baker's yeast that is found mainly when the small bowel is involved in Chron's disease. Nowadays ASCA is regarded more as a marker of increased gastrointestinal permeability than as a serological autoimmune marker of intestinal inflammatory disease. ASCA levels (anti-Saccharomyces Cerevisiae antibodies) are associated with increased intestinal permeability.

Objectives: At present we study the presence of ASCA in the serum of SSc patients aiming to know if there is increased intestinal permeability in this disease and if this is related to any clinical, demographic and treatment data.

Methods: Seventy four SSc patients and 57 healthy controls were studied for ASCA (IgG and IgA) positivity by ELISA. ASCA positivity was associated with demographic, clinical, severity index (Medsger score) and serological data in SSc patients.

Results: ASCA IgG levels were high in 26/74 (43.7%) patients from SSc group and 1/57 (5.2%) of controls (p<0.001); IgA ASCA was high in 12/74 (16.2%) from SSc group and 3/57 (1.7%) of controls (p=0.006). In univariate analysis IgG ASCA high levels associated positively with African background (p<0.0001) and