diagnosis of these patients are shown in Table 1. In the cluster analysis we could not identify clinical phenotypes, perhaps because of the small sample size. Only 50% of patients with myositis developed ILD. Regarding the final diagnosis, only 1 patient (5%) was diagnosed of scleromyositis. Besides detecting them in patients with SSc (39%) and idiopathic inflammatory myopathy (9%), anti-Ku Ab were detected in other SAD, the most frequent were systemic lupus erythematosus, rheumatoid arthritis (RA) and overlap syndrome of SSc + RA.

Table 1. Main clinical-analytical manifestations and final diagnosis of pacientes with anti-Ku Ab.

FINAL CLINICAL DIAGNOSIS

Scleromyositis: 1
Idiopathic inflammatory myopathy: 1
Systemic sclerosis (SSc): 6 (Pre-scleroderma: 3, limited SSc: 3).
Systemic lupus erythematosus: 2
Rheumatoid arthritis: 2
Overlap syndrome RA + limited SSc: 2
Primary Sjögren's syndrome: 1 (Secondary Sjögren's syndrome: 3)
Mixed connective tissue disease: 1
Polymyalgia rheumatica: 1
Undifferentiated connective tissue disease: 1
Acute hepatitis due to HEV: 1
Autoimmune thrombocytopenia (ITP): 1
Drug-induced fibrosing ILD: 1
Systemic graft versus host disease (GVHD) in a patient with acute lymphoblastic leukemia who received and autologous haematopoietic progenitor transplant: 1
Primary biliary cirrhosis: 1

CLINICAL MANIFESTATIONS (patients could have more than one):

Raynaud's phenomenon: 61% (14/23).
Inflammatory arthralgia/arthritis: 52% (12).
Lung involvement: 30.5% (7: NSIP 3, UIP 2, other patterns 2).
Serositis: 26% (6: pericarditis 4, pleuritis 1, pleuropericarditis 1).
Cardiac involvement: 26% (6: PHT by echocardiogram 3, myocarditis 2, arrhythmia 1).
Dry syndrome: 17% (4)
Myositis: 17% (4).
Esophageal involvement: 17% (4).
Autoimmune cytopenias: leucopenia: 17% (4) / thrombocytopenia: 13% (3).
Telangiectasias: 13% (3).
Photosensitivity: 13% (3).
Other: non-androgenic alopecia: 9% (2); sensory-motor polyneuropathy: 4.5% (1); Puffy hands: 4.5% (1); fever: 4.5% (1); lymphadenitis: 4.5% (1); cold sores: 4.5% (1), and retinal hemorrhage: 4.5% (1).

OTHER ASSOCIATED ANTIBODIES:

ANA: 91% (21/23)
anti Ro60/SSA Ab: 30.5% (7)
Anti Ro52 Ab: 30.5% (7)
Anti RNP Ab: 22% (5)
Anti-dsDNA: 17% (4)
Anti-La/SSB Ab: 17% (4)
Anticentromer Ab: 17% (4)
Anti Mi-2 Ab: 13% (3)
Other: anti Sm Ab: 9% (2); anticardiolipin Ab(IgG): 4.5% (1); PM/ScI: 4.5% (1); nucleosomes: 4.5% (1); ScI70: 4.5% (1); PL12: 4.5% (1); anti-U1-RNP: 4.5% (1) and NOR90: 4.5% (1).

Conclusion: Anti-Ku Ab are related to a great variety of SAD, without being a specific marker of any of them, nor being associated with any specific clinical manifestation. We couldn't confirm the existence of clinical phenotypes associated with the presence of these antibodies.

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AB0164

ACTIVITIES OF THE OXIDATIVE-RELATED ENZYMES IN SYSTEMIC SCLEROSIS

E. Mozgovaya¹, I. Zborovskaya¹, S. Bedina¹, A. Trofimenko¹, M. Mamus¹, E. Tikhomirova¹, S. Spitsina¹. ¹Research Institute of Clinical and Experimental Rheumatology named after A.B. Zborovskiy, Volgograd, Russian Federation

Background: The oxidative-related enzymes are involved in the pathogenesis of various stages of systemic sclerosis (SSc). SSc is a chronic autoimmune disorder that is intimately associated with vascular damage and therefore with chronic perfusion/reperfusion and oxidative organ injury. Mesenchymal cell activation in SSc is now also considered to be mediated primarily through oxidative burst. Regulation of oxidative stress by specific enzymes including several purine metabolism enzymes is likely to play an important role in SSc progression.

Objectives: to characterize interrelationships among circulating xanthine oxidase (XO), xanthine dehydrogenase (XDH), superoxide dismutase (SOD) activities and SSc activity.

Methods: The study was performed according to bioethical standards. 51 patients with verified SSc and 30 healthy controls were included in the study. The diagnosis was verified according to ACR/EULAR 2013 criteria. We assessed SSc activity in compliance with the original activity scale that is commonly used in Russia [Guseva N.G., 1993] and by the 2001 European Scleroderma Study Group Activity Index. XO (EC 1.17.3.2), XDH (EC 1.17.1.4), and SOD (EC 1.15.1.1) plasma activities were measured using spectrophotometric techniques as previously described [Dubinina E.E., 1986; Karpova O.V., 2006]. Results are expressed as mean±SD. The Mann-Whitney U test and Spearman's correlation coefficient were used for statistical analysis.

Results: Mean age of patients was 42.8±1.3 years, mean SSc duration was 7.9±0.7 years. Mean enzymatic activities in normal controls were 3.43±0.56 nmol/ml min (for XO), 5.19±0.71 nmol/ml min (for XDH), and 5.40±1.03 units (for SOD). The respective enzymatic activities in SSc group were 3.91±0.62 nmol/ml min, 7.10±0.71 nmol/ml min, and 7.10±2.19 units. All these mean activities were significantly higher in SSc patients comparing to healthy individuals (p<0.001). XO and XDH activities positively correlated with SSc activity (r=0.499, p<0.001, and r=0.741, p<0.001, respectively). The opposite but weaker trend was observed for SOD activity and SSc disease activity (r=-0.190, p=0.188).

Conclusion: SSc is characterized by an increase in the intensity of oxidative and antioxidant processes, more pronounced in high disease activity. A close relationship between function of prooxidant/antioxidant enzymes and some of the key SSc pathogenetic mechanisms, especially vascular disease and fibroblast activation, is widely considered. Overall increase of oxidative stress in patients with higher disease activity, as well as depletion of antioxidant capacity can be also linked with disturbance of purine metabolism through XO and XDH modulation. Pathogenetic influence of this imbalance can also be mediated through initial phase of neutrophil extracellular traps (NETs) formation, an eventual source of nucleoprotein containing autoepitopes.

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AB0165

THE ROLE OF CXCL4, CXCL8 AND GDF-15 IN SYSTEMIC SCLEROSIS

J.E. Oller Rodríguez¹, E. Grau García¹, R. Gonzalez Mazario¹, M. De la Rubia Navarro¹, C. Pávez Perales¹, S. Leal Rodríguez¹, I. Martínez Cordellat¹, F. M. Ortiz Sanjuan¹, E. Vicens Bernabeu¹, R. Negueroles Albuixech¹, I. Chalmeta Verdejo¹, C. Alcañiz Escandell¹, J. J. Frago-Gil¹, I. Cánovas Olmos¹, C. Nájera Herranz¹, L. Gonzalez Puig¹, J. Ivorra Cortés¹, D. Hervás Marín², J. A. Román Ivorra¹. ¹Rheumatology Service. HUP La Fe., Valencia., Spain; ²Biostatistics Unit. IIS La Fe, Valencia, Spain

Background: Systemic Sclerosis (SSc) is an autoimmune disease that can affect several organs and its mortality is fundamentally related to its pulmonary involvement.

It is mandatory to seek for biomarkers that help us with early diagnosis and that are also useful for predicting organic involvement, so that we can adjust the diagnostic and therapeutic approach.

Objectives: Our aim was to check if the presence of CXCL4, CXCL8 and GDF-15 is greater in the disease than in healthy population, and also their involvement in organic damage.

Methods: Observational and cross-sectional study, with a prospectively performed protocol, of patients diagnosed of SSc according to ACR/EULAR 2013 criteria. Demographic, clinical, analytical, activity (EUSTAR index), severity (Medsker scale and modified Rodnan index), health perception (SF36) and disability (HAQ and Cochin test) variables were collected. Moreover, Videocapillaroscopy (VCL) and Respiratory Function Test were made, as well High Resolution Lung Tomography and Echocardiography in order to describe pulmonary features. Serum levels of CXCL4, CXCL8 and GDF-15 were measured in SSc patients and in healthy controls.

Results: A total of 42 patients (95.4% women) were included, with an average age of 59.2 years. The median of years since diagnosis was 4, by 6 since the first non-Raynaud symptom. 20 patients were diagnosed with limited SSc, 20 patients diffusely and 2 patients with SSc without scleroderma. 42 healthy controls were also included.

We found significantly higher levels of GDF-15 in patients with SSc (P<0.001), without significant differences in CXCL4 and CXCL8 levels between patients with SSc and healthy controls.

The presence of GDF-15 was associated with diffuse SSc (P=0.009), pulmonary arterial hypertension (P=0.038), interstitial lung disease (P=0.004), decreased forced vital capacity (FVC), (P=0.002), high serum titres of antiScI70 (P=0.006), increased disease activity measured by EUSTAR index (P=0.001), as with capillary dilations in Capillaroscopy (P=0.015).

Moreover, we found an association between CXCL4 levels and the consumption of complement C3 fraction (P=0.008) and skin involvement (higher Rodnan modified score), (P = 0.001); not finding association with lung involvement or other features (spirometric or analytical changes, capillaroscopy or functional tests).

Attending to CXCL8, it was associated to consumption of the C4 fraction of complement ($P=0.013$) and the presence of tortuosities in capillaroscopy ($P=0.02$) with no other significant findings.

Conclusion: The presence of GDF-15 is associated with diffuse SSc, lung impairment, disease activity and changes in capillaroscopy. In addition, CXCL4 was only associated with skin involvement, while CXCL8 was not related to any organic damage in our patients.

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AB0166

IMMUNOGLOBULIN G DERIVED FROM PATIENTS WITH SYSTEMIC SCLEROSIS IMPRINTS A PRO-INFLAMMATORY AND PRO-FIBROTIC PHENOTYPE IN MONOCYTE-LIKE THP-1 CELLS

A. Dalmann¹, S. Murthy², M. Wannick², G. Eleftheriadis², A. Müller¹, D. Zillikens², H. Busch³, C. Sadik², G. Riemekasten¹. ¹University of Luebeck, Department of Rheumatology and Clinical Immunology, Luebeck, Germany; ²University of Luebeck, Department of Dermatology, Allergy, and Venereology, Luebeck, Germany; ³University of Luebeck, Luebeck Institute for Experimental Dermatology, Luebeck, Germany

Background: Regulatory IgG autoantibodies directed against diverse G protein-coupled receptors (GPCR), i.e. antibodies with agonistic or antagonistic activity are abundant in human serum. The serum titers of autoantibodies targeting angiotensin II receptor 1 (AT₁) and endothelin receptor A (ET_A) are specifically altered in autoimmune diseases such as systemic sclerosis (SSc). Disease-promoting mechanisms regulated by anti-AT₁ and anti-ET_A IgG are still elusive, but induction of pro-inflammatory and pro-fibrotic chemokines (CXCL8, CCL18) has been suggested to be one of them.

Objectives: To determine the cytokine and phospho-kinase profiles induced in monocyte-like cells by IgG derived from SSc patients (SSc-IgG) enriched with anti-AT₁ and anti-ET_A antibodies in comparison to IgG derived from healthy donors (IgG-HD).

Methods: A monocyte-like cell line (THP-1) was cultured *in vitro* and stimulated with IgG (1 mg/ml) derived from SSc patients or HD in the presence of various inhibitors/blockers for 24h. Then, supernatants were analyzed by a human cytokine/chemokine array. Data were analyzed using bio-mathematical tools such as generalized t-test including the robust regression method from R/Bioconductor package LIMMA. In addition, THP-1 cells were cultured *in vitro* and stimulated with IgG (1 mg/ml) derived from SSc patients or HD for up to 30 minutes. Thereafter, cell lysates were assayed for the kinome employing a human phospho-kinase array. To validate potential effects of transcription factor inhibition, release of CXCL8 and CCL18 into the supernatant was measured by Elisa.

Results: In general, SSc-IgG induced the release of most cytokines by THP-1 cells more pronouncedly than HD-IgG. The bio-mathematical analysis suggested that stimuli, responsible for the shift of the THP-1 cell cytokine profile, are more abundant in SSc-IgG than in HD-IgG. Based upon these findings a gene set enrichment analysis for transcription factors yielded the transcription factors NF- κ B, AP-1, and PRDM1 (Blimp-1) as putative major regulatory hubs for the response of THP-1 cells to SSc-IgG. Further, SSc-IgG altered the phosphorylation status of several proteins, indicative of an involvement of MAPK and/or JAK/STAT pathways. Interestingly, a role for AP-1 was also proposed by the inhibition of CXCL8 and CCL18 release following pretreatment of THP-1 cells with an AP-1 blocker.

Conclusion: Herein, we demonstrate that IgG of SSc patients, enriched with anti-AT₁ and anti-ET_A autoantibodies drives THP-1 cells towards a general pro-inflammatory and pro-fibrotic phenotype, which is reflected by broad changes in the secretome and kinome of these cells. Furthermore, our results highlight AP-1 as critical regulator of gene transcription of CXCL8 and CCL18 in a monocyte-like cell line.

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AB0167

TOFACITINIB AND NINTEDANIB MODULATE COLLAGEN FORMATION IN DERMAL FIBROBLASTS

A. S. Siebuhr¹, M. Karsdal², P. Juhl¹, A. C. Bay-Jensen¹. ¹Nordic Bioscience, ImmunoScience, Herlev, Denmark; ²Nordic Bioscience, R&D, Herlev, Denmark

Background: Dermal fibroblasts are responsible for the excessive extracellular matrix (ECM) formation observed in the skin of systemic sclerosis (SSc) patients and fibroblasts are therefore an obvious target for anti-fibrotic treatments. TGF β , PDGF and IL-6 are known to be central cytokines in systemic sclerosis. Nintedanib, a tyrosine-kinase inhibitor approved for treatment of idiopathic pulmonary fibrosis, did not show effect on dermal fibrosis only on pulmonary fibrosis in SSc patients with interstitial lung disease (ILD). Tofacitinib, as Janus kinase inhibitor, has shown to inhibit dermal fibrosis in mouse models and shown positive indications in patients.

Objectives: We investigated the direct effect of Nintedanib and Tofacitinib on ECM production from human dermal fibroblast using translational biomarkers of type I, III and VI collagens and fibronectin.

Methods: Primary healthy human dermal fibroblasts were grown in DMEM media containing 0.4% fetal calf serum, Ficol (to produce a crowded environment) and ascorbic acid for up to 17 days. The cells were stimulated with PDGF [3 nM] and/or TGF β [1 nM] in combination with Nintedanib [1 nM-10 μ M] treatment initiated at day 0 or 7 or Tofacitinib [3-100 nM] treatment initiated at culture start together. Media and treatments were changed twice a week. Non-activated cells (w/o) were used as control. Type I, III and VI collagen formation (PRO-C1, PRO-C3 and PRO-C6, respectively) and fibronectin (FBN-C) were evaluated by validated ELISAs (Nordic Bioscience). Statistical analysis included 1-way and 2-way ANOVA, AUC and Mann-Whitney U-test.

Results: PDGF significantly increased collagen type III and VI formation and collagen type I formation minimally. PDGF did not induce changes in fibronectin levels. TGF β increased collagen type I and VI formation but did not induce formation of collagen type III. TGF β increased fibronectin levels, where PDGF did not. Nintedanib (≥ 100 nM) added either from day 0 or 7 reduced PDGF induced collagen type III and VI formation to the levels of w/o throughout the remainder of the study. In TGF β treated fibroblasts, Nintedanib added either from day 0 or 7 reduced collagen type I and VI formation. The fibronectin levels were dose-dependently reduced by Nintedanib. The biomarker levels were at study end at the level of w/o. Nintedanib at a concentration of 1 μ M and higher significantly decreased the biomarker levels. Nintedanib (≥ 100 nM) in fibroblasts stimulated with both TGF β and PDGF significantly reduced collagen type I, III and VI collagen and fibronectin.

A Tofacitinib concentration of 100 nM was toxic to the dermal fibroblasts as the cell viability was minimal at culture end. However, the viability of Tofacitinib (100 nM) in combination with TGF β was decreased at study end, but only to half the viability of untreated cells. Tofacitinib dose-dependently decreased the TGF β induced type I and III collagen formation and fibronectin in the dermal fibroblasts. Tofacitinib (100 nM) decreased the level of collagen type I and III formation to the level of w/o, where as the level of fibronectin was lowered by 80 % of TGF β . Tofacitinib as low as 12.5 nM significantly lowered the collagen type I formation and fibronectin (both $p < 0.05$) and Tofacitinib of 25 nM decreased collagen type III formation significantly ($p < 0.0001$).

Conclusion: Tofacitinib decreased the formation of the collagens and fibronectin. Nintedanib inhibited ECM production differently in PDGF and TGF β induced dermal fibroblast, but in the combination of TGF β and PDGF Nintedanib significantly decreased the ongoing fibrosis. In PDGF induced fibrosis, Nintedanib acted as an on-off switch, whereas the inhibition was dose-dependent in TGF β induced fibrosis. This cell study indicates that Nintedanib and Tofacitinib inhibits collagen production in dermal fibroblasts.