Abstract THU0325 – Figure 2. Smooth muscle actin (α -SMA) expression by immunohistochemistry in TGF- β 1 treated fibroblasts. Fibroblasts were treated with 1 ng/ml TGF- β 1. Detection was performed with 2 μ g/ml anti α -SMA. **A)** α -SMA expression observed with a 10x magnification. Bar 100 μ m. **B)** 20x magnification. Bar 50 μ m. **C)** 40x magnification. Bar 20 μ m.

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THU0326 ANALYSIS OF PHOSPHATIDYLINOSITOL 3-KINASE PATHWAY IN B CELL ACTIVATION OF SYSTEMIC SCLEROSIS PATIENTS

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Background: B cell activation is an early event in the development of systemic sclerosis (SSc) as transcriptome profiling identified local B-cell activation in early diffuse cutaneous (dc) SSc skin biopsies. Autoantibody production is a widely investigated function of B cells in SSc but less attention has been devoted to the role of innate immune molecules and their receptors, like Toll-like receptors (TLRs). The classical B cell activation downstream of BcR and CD19 co-receptor engagement involves phosphatidylinositol-3 kinases (PI3K) signaling pathway that integrates the effects of multiple co-stimulatory receptors.

Objectives: Our goal was to examine PI3K signaling by investigating the mRNA expression of 92 pathway related genes in B cells from early dcSSc patients. Akt is a central element of PI3K pathway, but it can also converge into innate receptor mediated pathways such as mTOR and MAPK. Thus for functional relevance we also analyzed the phosphorylation of Akt, S6 and NF- κ B in B cells from early dcSSc patients.

Methods: Twenty-one patients with early dcSSc were enrolled with disease duration of 2.0 ($\pm$ 1.2) years based on the date of the first non-Raynaud's symptom. 30% of patients received immunosuppressive therapy. Peripheral blood CD19+ B cells were purified from dcSSc patients and age- and sex-matched healthy controls (HC, n=15). mRNA expression of 92 B cell specific PI3K pathway related genes was measured using a Taqman qPCR array and was validated by individual qPCR. Isolated B cells were activated through BcR using anti-IgG/M antibody or anti-CD180 antibody, and their combination followed by flow cytometric analysis of phospho-Akt (S473), phospho-S6 (S235/S236) and phospho-NF- κ B p65 (S529) positive cells.

Results: Analyzing the expression of 92 PI3K pathway associated genes we found altered expression of molecules playing a role in alternate B cell activation and innate signaling. The expression of both IL-4 receptor and osteopontin (SPP1) was upregulated in B cells of untreated dcSSc patients, but became downregulated upon immunosuppressive therapy. Innate molecules like TLR4 and complement component 3 remained highly upregulated and the downregulation of CD180 was not inhibited by immunosuppressive treatment, thus may preserve the activated state of B cells in dcSSc. We aimed to model B cell activation via TLR4 and CD180 but LPS and endogenous ligands of TLR4 failed to activate isolated B cells. Since there are not known ligands of CD180, we investigated the effects of anti-CD180 itself and also its combination with BcR mediated stimulation of B cells. Analyzing the phosphorylation of the PI3K pathway-associated molecules revealed an impaired responsiveness of dcSSc B cells to anti-CD180 alone, and the combination of anti-CD180 and anti-Ig induced different phosphorylation patterns of Akt, S6 and NF- κ B molecules in dcSSc and healthy controls (HC).

Conclusion: Analysis of B cells from early dcSSc patients revealed that B cells possess molecular changes in PI3K pathway that involves innate immune components. Gaining new insight into innate B-cell activation in SSc could be helpful for finding new therapeutic targets.

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THU0327 ANTIBODIES AGAINST EXTRACTABLE NUCLEAR ANTIGENS (ENA) IN SCLERODERMA ARE NOT ONLY DIAGNOSTIC AND PROGNOSTIC TOOLS, BUT PATHOGENETIC REGULATORS INDUCING A PROFIBROTIC PHENOTYPE IN CULTURED SKIN FIBROBLASTS

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Background: The importance of systemic sclerosis (SSc) autoantibodies for diagnosis has become increasingly recognized, as evidence by incorporation into the 2013 ACR/EULAR clinical classification criteria (1). Clear prognostic and phenotypic associations with cutaneous subtype and internal organ involvement have been extensively described (2). However, little is known about the potential of those autoantibodies to exert a direct pathogenetic role in the disease (3).

Objectives: The aim of the study is to assess the potential pathogenetic role of anti-topo isomerase I (Topo-I) and anti-centromeric protein B (Cenp B) autoantibodies to induce pro-fibrotic markers in dermal fibroblasts.

Methods: Dermal fibroblasts were isolated from unaffected and affected skin samples of (n=10) limited cutaneous SSc (LcSSc) patients, from affected skin samples of (n=10) diffuse cutaneous (DcSSc) patients and from (n=20) healthy subjects. Fibroblasts were stimulated with serum-isolated fractions of Topo-I, Cenp B and control IgGs in ratios 1:100 and 1:200 for 24 and 48 hours. Cells were also incubated with 10% SSc Topo-I⁺, Cenp B⁺ whole serum and with 10% control serum for 24 and 48 hours. Viability was assessed by MTT test, while apoptosis was assessed by flow cytometry. Activation of pro-fibrotic genes ACTA2, COL1A1 and TAGLN was evaluated by quantitative-real-time-PCR (qPCR), while protein levels alpha-smooth-muscle actin (α -SMA), type-I-collagen (Col-I) and SM22 were assessed by immunocytochemistry (ICC).

Results: MTT test showed that Cenp B ($p<0.05$) and with more extent Topo-I ($p<0.01$) IgGs reduced unaffected LcSSc and control fibroblast viability in a time- and dilution-dependent manner compared to control IgGs. Similar results were obtained with Cenp B⁺ ($p<0.05$) and Topo-I⁺ ($p<0.01$) sera compared to control sera. Flow cytometry analysis revealed that both Cenp B/Topo-I IgGs and Cenp B⁺/Topo-I⁺ sera induce apoptosis in unaffected LcSSc and control fibroblasts only, while affected LcSSc/DcSSc fibroblasts showed apoptosis resistance. qPCR showed that basal levels of pro-fibrotic markers ACTA2, COL1A1 and TAGLN were upregulated in affected LcSSc/DcSSc fibroblasts compared to LcSSc unaffected and to control ones. Stimulation with Cenp B and Topo-I IgGs and with Cenp B⁺ and Topo-I⁺ sera statistically increased all the profibrotic markers compared to control IgGs ($p<0.05$) and to control sera ($p<0.05$). ICC proved that α -SMA, Col-1 and SM22 levels were upregulated in a time- and dilution-dependent manner in unaffected LcSSc and control fibroblasts upon stimulation with Cenp B and Topo-I IgGs and with Cenp B⁺ and Topo-I⁺ sera, while they remained stably high in affected LcSSc/DcSSc fibroblasts.

Conclusion: This study demonstrates the pathogenetic role of antibodies targeting Topo-I and Cenp B to directly induce pro-fibrotic activation of fibroblasts. Therefore, besides the diagnostic and prognostic use of those autoantibodies in SSc, these data justify the importance of therapeutic use of immunosuppressive drugs in the early stages of the disease.

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THU0328

DO PATIENTS WITH SYSTEMIC SCLEROSIS HAVE ULTRASONOGRAPHIC MODIFICATIONS OF SALIVARY GLANDS SUGGESTIVE OF SJOGREN SYNDROM?

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Background: Modifications in ultrasonographic aspect of major salivary glands have been reported in patients with primary Sjogren Syndrome (pSS) with good diagnostic accuracy. Sicca symptoms are frequently observed in Systemic sclerosis (SSc).

Objectives: To assess the ultrasonographic echostructure of major salivary glands in patients with SSc and compare the modifications with those of patients with pSS or controls with sicca symptoms.

Methods: We performed a monocentre case-control study between 2014 and 2017 in the university hospital of Clermont-Ferrand (France). Patients with SSc and pSS were fulfilling the American College of Rheumatology/European League against Rheumatism (ACR/EULAR) 2013 and the ACR 2012 classification criteria respectively. Controls patients were complaining of sicca symptoms but did not meet the ACR 2012 criteria. Bilateral parotid and submandibular glands ultrasound (US) were performed in all patients by the same operator blinded to the diagnosis. Inhomogeneity of each of the 4 major salivary glands in B-Mode was graded using the Jousse-Joulin scoring system (scale of 0 to 4) as previously described.[1] The highest grade among the 4 glands was retained as suggestive of Sjogren Syndrome if ≥ 2 .

Results: A total of 108 patients were included: SSc (n=25), pSS (n=48) and controls (n=35). Among the 48 patients with pSS, 12 were receiving hydroxychloroquine, 4 an immunosuppressor, 77% had antinuclear antibodies at a significant level ($\geq 1/640$), 26 (54%) anti-SSA antibodies, 14 (29%) anti-SSB antibodies, 40/45 (89%) had a labial salivary biopsy suggestive of pSS (Chisholm and Mason score ≥ 3). Comparing the pSS and the control groups, performance of a US echostructure grade ≥ 2 for the diagnosis of pSS was good: Se=75%, Sp=91.4%, positive predictive value= 92.3%, negative predictive value= 72.7%.

Among the 25 SSc patients, 7 had immunosuppressor therapy, 8 had a localized SSc. Shimer's test ≤ 5 mm in 5 minutes was present in 9/18 (53%), unstimulated salivary flow ≤ 0.1 mL/minute in 8/14 (57.1%).

In the SSc group, 12 patients had an US echostructure grade 0, 6 grade 1, and 7 patients (28%) had an US echostructure grade of ≥ 2 : US score=3 (n=5), US score=4 (n=2). Anti-SSA antibodies were found in 1/7 patients with an US echostructure grade ≥ 2 and 2/18 patients with US echostructure grade 0 or 1.

	SSc group (n=25)	pSS group (n=48)	Control group (n=35)
Age, mean $\pm$ SD, years	64.8 $\pm$ 9.3	62.1 $\pm$ 12.3	55 $\pm$ 15
Female sex, %	88	96	97.2
Disease duration, mean $\pm$ SD, years	16.7 $\pm$ 11.1	10.5 $\pm$ 7.7	Not applicable
US Score ≥ 2 , n (%)	7 (28)	36 (75)	3 (8.6)

Conclusion: Nearly one third of patients with SSc have US echostructure changes suggestive of Sjogren Syndrome regardless of the presence of anti-SSA antibodies.

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THU0329

RED FLAG SIGNS OF SYSTEMIC SCLEROSIS ARE PREVALENT IN SUBJECTS WITH RAYNAUD'S PHENOMENON IN THE GENERAL POPULATION AND MAY BE A PROXY FOR LUNG INVOLVEMENT

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Background: Pulmonary involvement in systemic sclerosis (SSc) is very difficult to treat when diagnosed too late. Therefore, in order to optimally use "the window of opportunity" more attention should be given to the early identification of SSc. To the best of our knowledge, no studies exist that have structurally assessed the epidemiology of red flag signs and potential signs of pulmonary involvement in patients with Raynaud's phenomenon (RP).

Objectives: To assess the prevalence of red flag signs in participants with RP. Moreover, we aim to investigate the occurrence of pulmonary signs and symptoms in participants with red flag signs.

Methods: We retrospectively analyzed data from the LifeLines Cohort Study, which is a large population-based cohort study in the Northern parts of the Netherlands. A total of 74011 participants completed the connective tissue disease questionnaire. The presence of RP and red flag signs for SSc (i.e., puffy fingers, skin thickening distal, skin thickening proximal, and pitting scars) were obtained. Patients were classified as having red flag signs by the presence of at least one red flag sign in addition to RP. In addition, patient characteristics, self-reported pulmonary complaints, spirometry (screening for interstitial lung disease (ILD)), and uric acid (global screening for pulmonary arterial hypertension (PAH)) were also obtained. Three groups of participants were formed, namely: participants with RP and red flag signs (n=981), participants with RP without red flag signs (n=2946), and participants without RP and without red flag signs (n=70037).

Results: The prevalence of red flag signs was 5 fold higher in participants with RP, as compared to the non-RP group (RP 25% [23.7-26.4], non-RP 5% [4.9-5.2], $p<0.001$). A total of 413(42.1%) of the participants with RP and red flag signs reported dyspnea, which was 1.5 fold higher than in those with RP but without red flag signs, and two-fold higher compared to participants without RP and without red flag signs ($p<0.001$). Moreover, dyspnea in rest and after exertion was more prevalent in participants with RP and red flag signs ($p<0.001$). In addition, participants with RP and red flag signs more frequently reported to suffer from pulmonary fibrosis ($p<0.001$, table 1), and had the lowest forced vital capacity, as compared to the other groups ($p<0.001$). Conversely, uric acid was not found to be elevated in participants with RP and red flag signs.

Conclusion: This unselected cohort study from the general population demonstrates that the prevalence of red flag signs in subjects with RP may be as high as 25%. Potential signs and symptoms of pulmonary complaints are more prevalent in participants with RP who also reported red flag signs. This could indicate an increased risk of pulmonary involvement (i.e., ILD and PAH) in RP patients with red flag signs, although additional specific tests are mandatory to substantiate definite disease.

Abstract THU0329 – Figure 1. Prevalence of red flag signs in participants with and without Raynaud's phenomenon.

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