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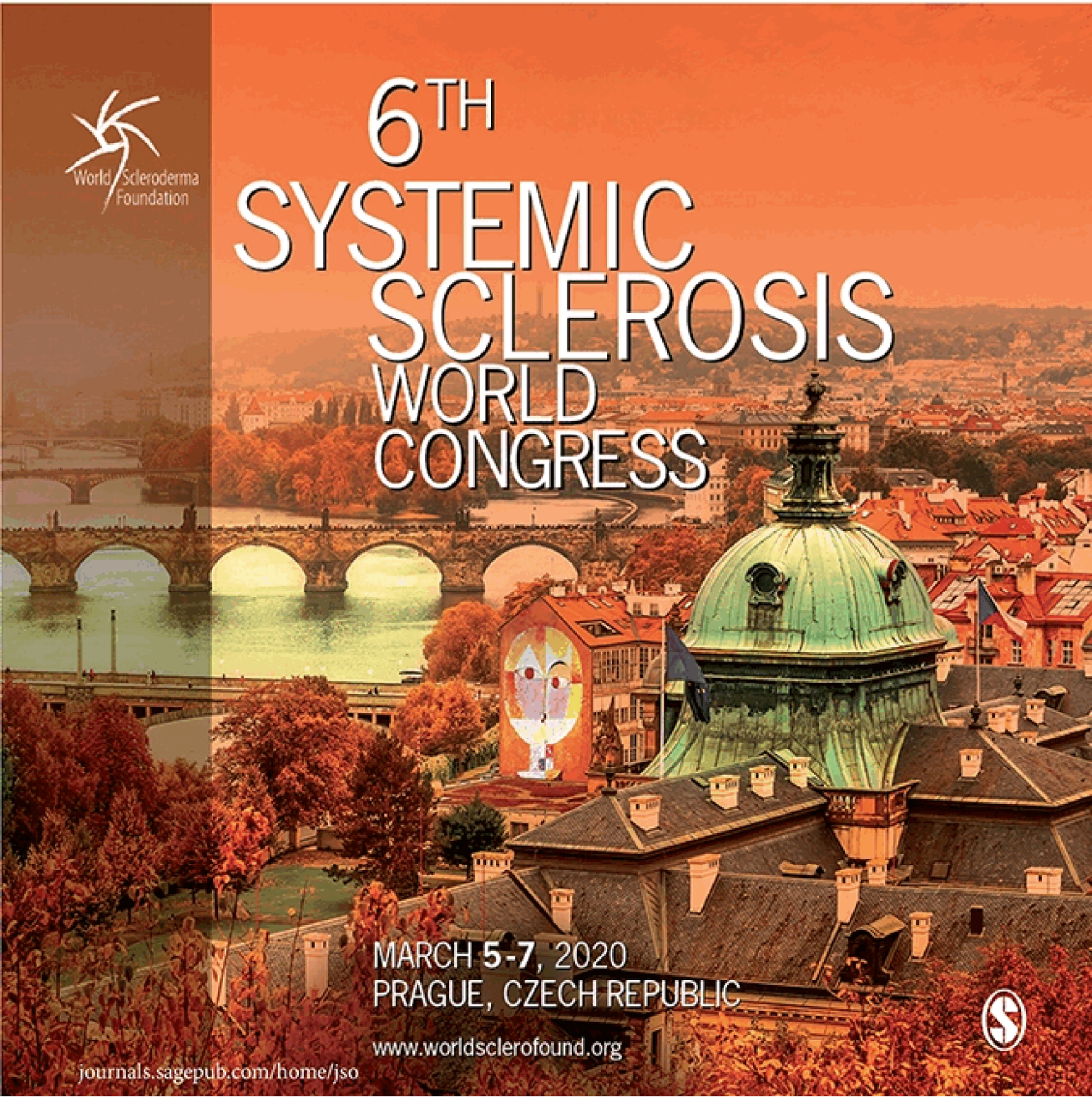

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**Introduction:** SSc patients contain stimulating antibodies targeting Platelet-derived growth factor receptor alpha (PDGFRalpha), which represents the main entry site for the adeno-associated virus type 5 (AAV5). We investigated the presence of AAV5 DNA in the lung and peripheral blood of SSc patients, and its relationship with anti-PDGFRalpha stimulating antibodies

**Material and Methods:** In silico molecular docking was performed to predict the binding between the three-dimensional structures of monomeric human PDGFRalpha and AAV5 capsid monomeric subunit. A surface plasmon resonance (SPR) assay was performed to validate in vitro the in silico prediction of the possible molecular complex between AAV5 and PDGFRalpha. The PDGFRalphaKO (by CRISP-CAS9 technology) human alveolar basal epithelial cell line A549, was used to assess the role of PDGFRalpha for AAV5 transduction. Bronchoalveolar lavage of 66 SSc patients and 77 controls affected by conditions other than SSc was analyzed by PCR for the presence of AAV5 DNA. The presence of the virus in the lung was also assessed by in situ hybridization, immunohistochemistry and confocal microscopy to demonstrate colocalization of PDGFRalpha and AAV5. Molecular docking, SPR, and immunoprecipitation studies, were performed to demonstrate the binding of anti-PDGFRalpha antibodies to AAV5.

**Results:** AAV5 in silico interacts with the extracellular domains of PDGFRalpha and SPR assay showed that the AVV5 capsid monomer binds PDGFRalpha with high affinity. Deletion of PDGFRalpha by CRISP-CAS9 in A549 cells inhibits significantly the transduction efficiency. AAV5 genomic sequences were found in 71.2% of SSc patients and in 28.5% of controls ($p<0.0001$). AAV5 was present in alveolar epithelial cells by immunohistochemistry and in situ hybridization and co-localized with PDGFRalpha by confocal microscopy. Both total SSc-IgG and human monoclonal anti-PDGFRalpha antibodies (Moroncini et al 2015) immunoprecipitated PDGFRalpha and AAV5 capsid from infected cells. Specific PDGFRalpha and AAV5 peptides recognize the monoclonal anti-PDGFRalpha antibodies.

**Conclusions:** The present study demonstrates that AAV5 is present in the lung and blood in a significant fraction of SSc patients, and recognizes stimulatory anti-PDGFRalpha antibodies. AAV5 contributes to the pathogenesis of systemic sclerosis by eliciting adaptive immunity targeting the PDGFRalpha complex.

## CO.31

## IMMUNE MECHANISMS OF DISORDER IN THE WORK OF THE BODY'S ANTIOXIDANT SYSTEM IN THE PATHOGENESIS OF LUNG DAMAGE IN SYSTEMIC SCLERODERMA

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**Introduction:** Oxidative stress plays an important role in the pathogenesis of lung injury and pulmonary fibrosis in patients with systemic scleroderma (SSc), which is considered to be potentially the most serious visceral lesions in this disease.

Objective: to study the clinical and laboratory features of lung disease and the role of antioxidant enzymes in the development of organ pathology in SSc.

**Material and Methods:** The study included 83 patients with a reliable diagnosis of SSc (97.6% of women, mean age 50.3±11.9, disease duration 8.3±7.1 years) and 30 healthy individuals. The initial stage of the disease was detected in 13.3% of cases, the developed one - in 75.9%, the terminal - in 10.8%.

The patients were assigned to clinical, laboratory, functional and instrumental research methods with the obligatory radiography and/or computer tomography of the chest organs and assessment of the function of external respiration.

Antibodies of the IgG class to antioxidant enzymes (glutathione peroxidase, glutathione reductase, superoxide dismutase) were determined in blood serum by the standard ELISA test using an immobilized antigenic form of the corresponding enzyme.

**Results:** Lung damage was observed in 66 (79.5%) patients with SSc and was mainly represented by interstitial pulmonary fibrosis (mainly the basal sections) - 60% of cases and pulmonary hypertension (according to echocardiography) - 6% of cases. Restrictive breathing disorders (reduced forced lung capacity below 80% of the proper values) were noted in 38 people (57.6%). Imbalance of the oxidative-antioxidant was observed in the early stages of the disease along with inflammation, disruption of the immune system (overproduction of various autoantibodies) and the development of vascular abnormalities with the subsequent spread of fibrosis.

The level of all antibodies studied in SSc was increased in comparison with healthy individuals ($p<0.005$). A significant decrease in the activity of glutathione peroxidase ($p=0.008$) and superoxide dismutase ($p=0.042$), an increase in the level of antibodies to glutathione reductase ($p=0.04$) and

glutathione peroxidase (p=0.037) were observed in patients with respiratory failure with lung damage. Also in the group of patients positive for antibodies to superoxide dismutase the signs of lung damage were found statistically significantly more often ($\chi^2$ with Yates correction = 3.47, p=0.048).

**Conclusions:** Glutathione transferase and superoxide dismutase are believed to play a key protective role in the pulmonary matrix. Antibodies to antioxidant enzymes can inhibit the extracellular activity of enzymes and reduce the possibility of antioxidant defense of the body, especially due to mechanisms that protect the lungs from injuries, inflammation and fibrosis.

CO.32

### TGFBRII-FC REDUCES FIBROSIS AND IMPROVES LUNG FUNCTION IN FOS-RELATED ANTIGEN-2 TRANSGENIC MICE, A MODEL OF SYSTEMIC SCLEROSIS

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**Introduction:** Interstitial lung disease (ILD) is the leading cause of death for patients with systemic sclerosis (SSc). In SSc-ILD, interstitial lung fibroblasts undergo phenotypic conversion to αSMA-expressing myofibroblasts that deposit abnormal levels of extracellular matrix, leading to fibrosis and a decline in pulmonary function. TGF-β1 is a potent inducer of epithelial-to-mesenchymal transition and fibroblast-to-myofibroblast activation, two key cellular events leading to the uncontrolled deposition of fibrillar collagen, the hallmark of lung fibrosis. TGF-β1 is upregulated in lung and skin tissue of SSc patients and in animal models of lung fibrosis.

**Material and Methods:** We used TGFβRII-Fc, a selective ligand trap that neutralizes TGF-β1 and -β3, to investigate its protective effects in Fos-related antigen-2 transgenic (Fra2Tg) mice. Fra2 is upregulated in fibrotic lung tissue of SSc-ILD patients and in skin of SSc patients. Fra2Tg mice spontaneously develop severe, progressive pulmonary fibrosis, leading to death by respiratory failure at a median age of 17 weeks. In addition, Fra2Tg mice develop fibrosis and peripheral vasculopathy in skin. Hence, this model closely replicates the important pathological features of SSc in lung and skin.

**Results:** TGFβRII-Fc significantly inhibited TGF-β1-induced myofibroblast activation and epithelial-to-mesenchymal transition in vitro, as demonstrated by reduced expression of pro-fibrotic and pro-inflammatory genes ($p < 0.001$). Over the course of the in vivo study, Fra2Tg mice displayed a progressive increase in pulmonary collagen deposition, as determined by the fractional area of lung tissue stained by picrosirius red, and a significant decline in lung function as determined by plethysmography. Treatment of Fra2Tg mice with TGFβRII-Fc (10 mg/kg, s.c.) twice weekly for 8 weeks (starting at 8 weeks of age) completely inhibited pulmonary collagen deposition and significantly reduced (by 35%, $p < 0.05$) the decline in pulmonary function compared to vehicle. TGFβRII-Fc also significantly reduced dermal collagen content ($p < 0.05$) and dermal thickness in Fra2Tg mice and attenuated loss of body weight ($p < 0.01$). In addition, TGFβRII-Fc treatment inhibited pulmonary infiltration of inflammatory cells.

**Conclusions:** TGFβRII-Fc significantly reduces interstitial lung fibrosis, attenuates the decline in pulmonary function, and markedly downregulates expression of fibrosis-associated genes in a preclinical model of SSc-ILD. TGFβRII-Fc treatment may provide an effective therapeutic option for SSc patients with interstitial lung disease.

## SESSION 8 – SKIN

CO.33

### BIOSAMPLES FROM AT RISK SSC PATIENTS SHOW CLASSIC PATHOLOGICAL SIGNS OF SCLERODERMA: OPPORTUNITY FOR A DIAGNOSIS OF PRE-CLINICAL SSC

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**Introduction:** The VEDOSS study has recently indicated that more than 80% of patients affected by Raynaud's phenomenon (RP) and specific SSc auto-antibodies + capillaroscopy changes satisfied ACR/EULAR 2013 criteria within 5 years. These data suggest that there is a window of opportunity for early detection of SSc in these patients. Here we aimed to determine whether sera, skin biopsies and skin fibroblasts cultured from these patients showed any biomarker sign of SSc.

**Material and Methods:** Fifty-nine at risk patients identified by having RP and SSc auto-antibodies or capillaroscopy pattern (or both) were enrolled in the national inception cohort (Kennedy Cohort). Sera were tested for IFN inducible chemokines (CXCL-9,10 and 11 and CCL2, 8 and 19) and biomarker of extracellular matrix turnover (ELF test), all previously shown to be increased in SSc. Further, two 3mm skin biopsies were taken from the forearms from 3 ACA+ve (anti-centromere antibodies), 3 SCL70+ve patients. One biopsy was subjected to histology