

**AB0682** **CIRCULATING FIBROCYTES IN LIMITED CUTANEOUS SYSTEMIC SCLEROSIS PATIENTS: CORRELATION WITH DERMAL THICKNESS**

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**Background:** Systemic sclerosis (SSc) is characterized by early skin impairment (1,2) and the modified Rodnan skin score (mRSS) is the validated method to assess the severity of this impairment. Whilst skin high frequency ultrasound (US) is a relatively new technique to measure dermal thickness (DT) (2-4).

Recent findings have reported the important contribution circulating fibrocytes make in the early stage of dermal repair and fibrosis. Indeed, as fibrocytes may be an important source of activated fibroblasts/myofibroblasts, it is reasonable to presume that they could be responsible for the increase in these cells observed in the tissue of SSc patients (5-7).

**Objectives:** The aim of this study was to identify any correlation between the modified Rodnan skin score (mRSS), dermal thickness (DT), measured by skin high frequency ultrasound (US) and the percentage of circulating fibrocytes in patients with limited cutaneous systemic sclerosis (lcSSc).

**Methods:** After obtaining approval and written informed consent and the Ethics Committee approval 8 lcSSc patients (7 females, 1 male) and 5 (4 females, 1 male) age-matched healthy volunteers (CNT) were enrolled. The lcSSc patients fulfilled the 2013 ACR/EULAR criteria for SSc (8). DT was evaluated by both mRSS and US (18 and 22 MHz probes), in all SSc patients and CNT, in the standard 17 skin areas evaluated by mRSS. The percentage of circulating fibrocytes was obtained by isolating them from the peripheral blood mononuclear cells (PBMCs) in all lcSSc patients and CNTs. Non-parametric tests were used for the statistical analysis.

**Results:** The percentage of circulating fibrocytes was positively correlated with DT-US, evaluated by the 22 MHz and the 18 MHz probes ( $p=0.04$  and  $p=0.03$ , respectively) and mRSS ( $p=0.04$ ) in lcSSc patients. Conversely, there was no correlation between these parameters in the CNT group ( $p>0.05$ ).

**Conclusion:** The study demonstrates a significant relationship between DT, evaluated by both mRSS and US and the percentage of circulating fibrocytes in lcSSc patients. This observation may well support the hypothesis that circulating fibrocytes make a crucial contribution to skin fibrosis progression.

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**AB0683** **ROLE OF ELASTIN AND ELASTASE ANTIBODIES IN DEVELOPMENT OF PNEUMOSCLEROSIS IN PATIENTS WITH SYSTEMIC SCLERODERMA**

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**Background:** Disturbed elastin metabolism, emerging pathological soluble isoforms followed by triggering of autoimmunity show that the biopolymer under study is associated with the pathogenesis of polyorganic lesions in systemic sclerosis. Elastin catabolism is promoted by elastase, an enzyme with a broad-range substrate specificity. It is the balance of a dynamic elastin-elastase system that determines physiological functioning of organs and tissues containing elastic fibres: the skin, ligaments, lungs,

and vascular walls. Antibodies to elastin and elastase are unique predictors of development of pulmonary conditions in systemic scleroderma, which is now considered to be one of risk factors for pulmonary lesion.

**Objectives:** Developing highly sensitive markers of pulmonary remodeling at early stages of systemic scleroderma with participation of humoral immunity to the elastin-elastase system in the development of pulmonary lesion in systemic scleroderma requires further detailed study.

**Methods:** Patients for the study were selected from the department of rheumatology at the Emergency Care Municipal Hospital 25 in the city of Volgograd. The main group incorporated 42 persons with the diagnosis of systemic scleroderma verified by ACR/EULAR diagnostic criteria of 2013, without any exclusion criteria. There were 11 men and 31 women aged 22-72 among the systemic scleroderma patients. The mean age of patients was 44.1±15.4. The control group incorporated 30 healthy donors at the Volgograd Regional Blood Transfusion Station. Antibodies to elastin and elastase were determined in the blood serum by indirect enzyme immunoassay utilizing insoluble forms with enhanced antigen content according to the original technique by Gontar et al (1990).

**Results:** Compared with the control group, patients with systemic scleroderma showed a considerably higher rate of antibodies to elastase (52%) and elastin (38%). The upper normal level of antibodies to elastin was in the range of 0.131 absorbance units, of antibodies to elastase – 0.131 absorbance units. The mean value of elastin antibodies in the blood of systemic scleroderma patients was 0.125 ± 0.068 absorbance units. The value of elastase antibodies was 0.143 ± 0.071 absorbance units. The elevated level of elastin and elastase antibodies in the patients was associated with pulmonary lesion (basal pulmonary fibrosis – 20 (47.6%), pulmonary hypertension – 11 (26.2%), and adhesive pleurisy – 7 (16.7%). In one half of cases the pulmonary lesion was asymptomatic, detected by accessory investigations like X-ray of chest organs, respiratory function study, EchoCG which were administered to assess the extent of abdominal organ inflammation and in differential diagnosis with cardiac insufficiency. The level of elastin antibodies in systemic scleroderma patients with pulmonary lesion was 0.149 ± 0.074 absorbance units, and of elastase antibodies – 0.146 ± 0.043 absorbance units, which was considerably higher than mean values in the control group.

**Conclusion:** By determining antibodies to elastin and elastase by the proposed technique of enzyme immunoassay at early stages of systemic scleroderma one can predict lesions of pulmonary parenchyma presenting with various clinical signs, and exacerbation of fibrosis processes.

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**AB0684** **DO WE DIAGNOSE ALL THE ANTISYNTHEASE SYNDROME?**

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**Background:** Antisynthetase syndrome (AS) is an autoimmune disease characterized by the presence of anti-aminoacyl tRNA-synthetase antibodies (anti-ARS) and clinical manifestations that may include interstitial lung disease (ILD), myositis, non erosive arthritis, Raynaud's phenomenon (RP), fever and mechanic's hands. Until the Connors diagnostic criteria appeared in 2010, these patients with anti-ARS antibody were classified as an idiopathic inflammatory myopathy, or if they did not present myositis, they remained undiagnosed.

**Objectives:** To identify patients with anti-ARS antibodies from our hospital since June 2015, when the immunology laboratory was inaugurated, and to verify if they fulfilled the AS criteria.

**Methods:** Retrospective observational study carried out in a regional hospital in Barcelona with a reference population of 260,000 inhabitants. All patients with any anti-ARS antibody (Jo1, PL7, PL12, EJ) positive were selected, determined by the Dot Blot Polymyositis/Scleroderma IgD D-Teck. We applied Connors diagnostic criteria and collected demographic, clinical and instrumental data, and types of antibodies.

**Results:** We identified 18 patients with anti-ARS antibody, 9 anti-Jo1 (53%), 4 anti-PL7, 3 anti-PL-12 and 1 anti-EJ. Of these, 13 had values of ANA > 1/80, 3 values of 1/80 and 1 negative. Other antibodies identified were anti-Ro52 in 4, anti-DNA in 4 and anti-Sc170 in 1. All patients except one met AS criteria. None of anti-DNA positive subjects met criteria for erythematosus systemic lupus or systemic sclerosis. The majority

were women (88%) with a mean age at diagnosis of 62 years (27-83 years), 16 Caucasians and 1 Asian. The clinical manifestations reported were: 5 myositis (29%), 11 ILD (6 BOOP and 5 NSIP) (65%), 6 arthritis (2 polyarthritis, 2 oligoarthritis, 1 palindromic, 1 shoulder girdle syndrome) (35%), 3 Raynaud's phenomenon (17.5%), 2 mechanic's hands (11%) and 6 fever (35%). Only one patient had the classic triad (myositis, arthritis, ILD, Jo1), 2 had myositis with ILD (Jo1), 1 myositis with arthritis (PL12), 1 ILD with arthritis (PL7), 7 ILD (Jo1, PL7, PL12), 3 arthritis (Jo1), 1 RP (Jo1) and 1 fever (EJ). Ten had a nailfold capillaroscopy performed, 7 of them had any alteration: 1 active systemic sclerosis pattern and 6 nonspecific (6 ramified capillaries and 3 microhemorrhages).

**Conclusion:** Our immunologically defined cohort has fewer clinical manifestations than described in clinically defined cohorts (AENAS group and EuroMyositis). Only 30% of patients had myositis and 70% had a single non-myositis clinical manifestation associated with anti-ARS antibody. Actually, it involved de-novo diagnosis of AS in half of them. In patients with suspected AS with low or negative ANA, antibody blot determination is definitive.

Nailfold capillaroscopy in undefined cases is a fundamental tool for diagnosing AS.

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#### AB0685 ACROOSTEOLYSIS AND BONE METABOLISM PARAMETERS DISTINGUISH FEMALE PATIENTS WITH LIMITED SYSTEMIC SCLEROSIS WITH AND WITHOUT CALCINOSIS: A CASE CONTROL STUDY

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**Background:** Calcinosis represents a late manifestation of limited systemic sclerosis (ISSc), inducing tissue damage and chronic calcifications. Bone metabolism studies in ISSc patients are rare in literature and there are few studies that analyzed clinical, laboratory and bone mineral density (BMD) parameters together.

**Objectives:** The aim of this study was to compare and analyze clinical aspects and laboratory parameters, including bone metabolism variables in female ISSc patients with and without calcinosis, paired by age, disease duration and body mass index (BMI).

**Methods:** Thirty-six female ISSc patients with calcinosis were compared to 36 female ISSc patients without calcinosis, matched by age, disease duration and BMI. Organ involvement, autoantibodies, BMD by DXA and laboratory parameters were analyzed. The past and current treatment modalities were also questioned. Statistical significance was considered if  $p \leq 0.05$ .

**Results:** Esophageal hypomotility, digital ulcers, and interstitial lung disease were the most frequent clinical manifestations of ISSc patients, present in similar frequency in both groups. Calcinosis was significantly associated with acroosteolysis (69% vs. 22%,  $p < 0.001$ ), higher modified Rodnan skin score (mRSS:  $4.28 \pm 4.66$  vs  $1.17 \pm 2.50$ ,  $p < 0.001$ ), higher 25OHD ( $24.46 \pm 8.15$  vs.  $20.80 \pm 6.60$  ng/ml,  $p = 0.040$ ) and phosphorus ( $3.81 \pm 0.41$  vs.  $3.43 \pm 0.45$  mg/dl;  $p < 0.001$ ) serum levels. 25OHD levels  $> 30$  ng/ml were also significantly more frequent in patients with calcinosis ( $p = 0.041$ ). ANA was positive in 89% in both groups. Anticentromere antibody was frequent (44% and 31%), while positive anti-Scl70 was rare in both groups. Regarding treatment, current use of corticosteroids was lower in patients with calcinosis compared to patients without calcinosis (8% vs. 28%;  $p = 0.032$ ). Osteoporosis was more frequent in the group with calcinosis (31% vs. 17%), although not statistically significant.

**Conclusion:** This study showed that ISSc patients with calcinosis can present a distinct clinic and biochemical profile when compared to a matched group without calcinosis. Presence of calcinosis in female patients with ISSc can be associated with acroosteolysis and higher serum levels of 25OHD and phosphorus when compared with patients without calcinosis paired by age, disease duration and BMI.

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#### AB0686 REDUCTION IN THE ABSOLUTE NUMBER OF PERIPHERAL REGULATORY T CELLS CORELATED TO CLINICAL FEATURES IN PATIENTS WITH SYSTEMIC SCLEROSIS

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**Background:** The role of regulatory T cells (Tregs) in the development of SSc has started being studied during the last decade with new aspects being disclosed continuously. Although there is a general agreement in the medical literature regarding the decreased functional capacity of circulating Tregs in SSc, the alteration of absolute number of Treg and Th17 cells as well as their associations with clinical characteristics of SSc are still unclear.

**Objectives:** The present study was to investigate the relationship between multiple system impairments, disease activity and the absolute number of CD4+CD25+FOXP3+ regulatory T cells (Treg cells) and Interleukin 17 producing T help cells (Th17) in peripheral blood of patients with systemic sclerosis (SSc).

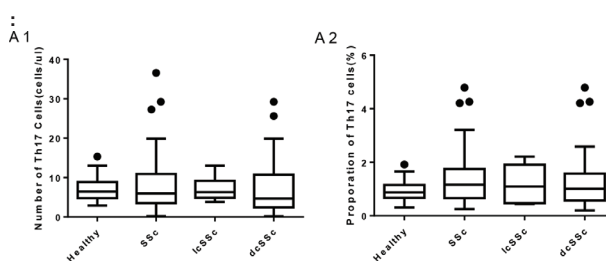
**Methods:** Clinical indicators were collected from total 54 patients with SSc (8 with localized SSc (lcSSc) and 46 with diffuse SSc (dcSSc)) who were divided into two groups, mainly with skin lesions (n=11) and with multiple system and organ besides skin lesions (n=43). Then According to the SSc EUSTAR index, we divided 54 SSc patients into the inactive (EUSTAR  $< 2.5$  points, n = 22 cases) and the active group (EUSTAR score  $\geq 2.5$  points, n = 32 cases). The levels of peripheral T lymphocyte were detected with flow cytometry.

**Results:** We found that SSc, especially dcSSc patients, had significantly fewer peripheral CD4Treg cells (both absolute number and proportion,  $p < 0.01$ ) than healthy controls (Figure 1). In addition, the absolute number of peripheral Treg cells was significantly reduced in SSc patients with multiple system impairments ( $P < 0.001$ ), however, the number of Th17 cells were significantly increased in SSc patients with simple skin involvement ( $P = 0.002$ ) (Figure 2). Also the absolute number of peripheral CD4Treg cells in active group with SSc decreased more significantly, thus causing an increase in the ratio of Th17 and Treg cells (Figure 2).

**Conclusion:** The reduced absolute number of peripheral Treg cells in SSc patients was contributed to the imbalance of Th17/Treg cells and involved in the pathogenesis of SSc, and may promote multiple system impairments and disease activity.

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**Figure 1.** Absolute number of peripheral Th17 and Treg cells of patients with SSc. (A1 and A2) Neither absolute number nor proportion of Th17 cells was increased. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . lcSSc: localized sclerosis, dcSSc: diffuse sclerosis.