Pavia, Fondazione IRCCS Policlinico San Matteo, Obstetrics and Gynaecology, Pavia, Italy

Background: Systemic Sclerosis (SSc) is one of the rheumatic diseases burdened with obstetrical complications. An Italian multicenter study showed that women with SSc have a higher-than-normal risk of intrauterine growth restriction, preterm delivery, very-low birth weight babies and pregnancy should be discouraged in patients with severe organ damage. However, with a multidisciplinary management, patients with SSc can have successful outcomes¹. Little is known about the pathogenesis of obstetrical complications, as studies on placenta are case reports or description of a few cases²⁻³.

Objectives: The aim of this study was to analyze the placental alterations with a focus on the role of inflammation in the pathogenesis of obstetrical complications in SSc, including the study of the atypical chemokine receptor 2 (ACKR2), involved in immune modulation and known to be highly expressed in circulating leucocytes in SSc patients⁴⁻⁶.

Methods: Eight SSc pregnant patients were compared with 16 patients with other rheumatic diseases (ORD) and 16 healthy controls (HC), matched for gestational age. Clinical data were collected. Placentas biopsies were obtained for histopathological analysis and immunohistochemistry for CD3, CD20, CD11c, CD68 and ACKR2. Frozen placenta samples from 4 SSc, 8 ORD and 8 HC were analyzed by qPCR for ACKR2 gene expression and proteins were extracted for multiplex assay for cytokines, chemokines and growth factors involved in angiogenesis and inflammation. Statistical analysis was performed with parametric or non-parametric tests depending on samples distribution.

Results: The number of placental CD3 ($p<0.05$), CD68 ($p<0.001$) and CD11c+ ($p<0.001$) cells was significantly higher considering the group of patients affected by rheumatic diseases (SSc+ORD) compared to HC. The SSc group alone did not show significance due to the lower sample size. No differences were observed between groups in terms of vascular alterations or fibrosis. The percentage of stained area for ACKR2 and the ACKR2 transcripts levels were comparable between groups. Hepatocyte growth factor (HGF), involved in angiogenesis, was significantly increased in the group of rheumatic diseases patients (SSc+ORD) compared to HC ($p<0.05$), while the chemokine CCL5 was significantly higher in SSc patients compared to patients affected by ORD ($p<0.05$) and to HC ($p<0.01$). CCL5 levels directly correlated with the number of all inflammatory cells considered and higher levels were associated to histological villitis ($p<0.01$).

Conclusion: The higher number of placental inflammatory cells and the alterations in the levels of HGF and especially CCL5 could play a role in the pathogenesis of the obstetrical complications in SSc. ACKR2 does not seem involved in the obstetrical complications of SSc.

References:

- [1] Taraborelli M, et al. Arthritis Rheum. 2012
- [2] Ibba-Manneschi L, et al. Ann Rheum Dis. 2010
- [3] Doss BJ, et al. Hum Pathol. 1998
- [4] Graham GJ. Eur J Immunol. 2009
- [5] Martinez de la Torre Y, et al. Proc Natl Acad Sci U S A. 2007
- [6] Codullo V, et al. Ann Rheum Dis. 2011

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.4379

SAT0281

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

R. Ross¹, I. Georgiou¹, A. Carriero^{1,2}, G. Abignano¹, C. Wasson¹, G. Migneco¹, A. Herrick³, C. Denton⁴, F. Del Galdo^{1,5}. ¹University of Leeds, Leeds Institute of Rheumatic and Musculoskeletal Medicine and Biomedical Research Centre, Leeds, United Kingdom; ²San Carlo Hospital of Potenza, Rheumatology Institute of Lucania (IRel) and Rheumatology Department of Lucania, Lucania, Italy; ³The University of Manchester, Division of Musculoskeletal & Dermatological Sciences (L5) Division of Musculoskeletal & Dermatological Sciences, Manchester, United Kingdom; ⁴University College London, Experimental Rheumatology, UCL Division of Medicine, London, United Kingdom; ⁵Chapel Allerton Hospital, NIHR Leeds Biomedical Research Centre, Leeds, United Kingdom

Background: The VEDOSS study has recently indicated that more than 80% of patients affected by Raynaud's phenomenon (RP) with specific SSc auto-antibodies and 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.

Objectives: Here we aimed to determine whether sera, skin biopsies and skin fibroblasts cultured from these patients showed any biomarker sign of SSc.

Methods: Fifty-nine at risk patients identified by having RP and SSc auto-antibodies or capillaroscopy pattern (or both) were enrolled in the Kennedy national inception 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 analysis, including haematoxylin and eosin staining and immunohistological staining for Collagen Type 1, alpha-SMA, Caveolin 1 and CD31 as endothelial marker. The other biopsy was used to explant fibroblasts cultures. mRNA and protein were isolated from primary fibroblasts and processed for RT-qPCR and western blotting analyses.

Results: Sera from at risk patients showed overall higher IFN inducible chemokines and ELF test ($P<0.05$) with bimodal distribution among patients. Skin biopsies from both ACA or SCL70+ve patients showed decreased number of CD31+ cells, increased number of myofibroblasts and increased collagen bundles within the dermis, as usually seen in SSc, compared to healthy controls. In vitro, fibroblasts from both ACA or SCL70+ve patients showed average 10-fold higher collagen mRNA levels and 31-fold increased collagen protein levels

compared to healthy control fibroblasts. Furthermore, fibroblasts from ACA or SCL70+ve patients showed limited TGF-beta induced increase in collagen and SMA expression, similar to SSc fibroblasts.

Conclusion: Although pilot in nature, this study suggests that patients "at risk" already show biomarker signs of SSc both in their sera, at skin biopsy and fibroblast level. Longitudinal studies on patients at this stage of pre-clinical disease may inform on the stratification strategies for imminent progression to clinical manifestations, and offer both insights on pathogenesis of clinical signs and a window of opportunity for delaying the onset clinical intervention trials.

Disclosure of Interests: rebecca ross: None declared, Ioanna Georgiou: None declared, Antonio Carriero: None declared, Giuseppina Abignano: None declared, Chris Wasson: None declared, Gemma Migneco: None declared, Ariane Herrick: None declared, Christopher Denton Grant/research support from: GlaxoSmith-Kline, CSL Behring, and Inventiva, Consultant of: Medscape, Roche-Genentech, Actelion, GlaxoSmithKline, Sanofi Aventis, Inventiva, CSL Behring, Boehringer Ingelheim, Corbus Pharmaceuticals, Acceleron, Curzion and Bayer, Francesco Del Galdo: None declared

DOI: 10.1136/annrheumdis-2020-eular.5666

SAT0282

ASSOCIATION BETWEEN A VARIANT OF THE SRP55 SPLICING FACTOR GENE AND SYSTEMIC SCLEROSIS IN AN ITALIAN POPULATION

E. Romano¹, M. Manetti¹, J. Kosalka-Wegiel², B. S. Fioretto¹, I. Rosa¹, E. Sticchi¹, S. Guiducci¹, S. Bellando-Randone¹, L. Ibba-Manneschi¹, M. Matucci-Cerinic¹. ¹University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy; ²Jagiellonian University Medical College, Department of Medicine, Cracow, Poland

Background: In systemic sclerosis (SSc), alternative splicing of the last exon (exon 8) of vascular endothelial growth factor (VEGF)-A pre-mRNA is a key element in the switch from proangiogenic to antiangiogenic VEGF-A isoforms. The mRNA-binding protein serine/arginine protein 55 (SRp55, also known as SFRS6) is a key regulatory splicing factor that promotes distal splice-site selection in the exon 8 region of VEGF-A pre-mRNA and subsequent upregulation of the exon 8b-containing VEGF₁₆₅ antiangiogenic isoform. Overexpression of both VEGF₁₆₅ and SRp55 has been implicated in SSc-related angiogenesis impairment and peripheral vascular damage. Moreover, differential splicing of the VEGF-A gene has been shown to be critical for development of pulmonary fibrosis. Of note, previous studies reported the lack of sequence variations in the VEGF-A alternatively spliced region, while a single nucleotide polymorphism (SNP) in the SRp55 gene (rs2235611) has been associated with susceptibility to disturbed ocular angiogenesis in proliferative diabetic retinopathy.

Objectives: This case-control pilot study examined the possible implication of SRp55 rs2235611 SNP in the genetic predisposition to SSc susceptibility and clinical phenotype.

Methods: A total population of 872 white Italian individuals (414 SSc patients, 458 controls) was studied. All patients were classified as limited and diffuse cutaneous SSc (lcSSc and dcSSc, respectively) and were clinically evaluated for the presence of autoantibodies (anticentromere, anti-Scl70 antibodies), pulmonary fibrosis and digital ulcers. The SRp55 rs2235611 SNP was genotyped by TaqMan Real-Time PCR.

Results: SRp55 rs2235611 genotype distribution and allele frequency were similar in SSc and healthy controls, though a trend toward significance was observed for genotype distribution ($p=0.07$). The SRp55 rs2235611 AA genotype significantly influenced the predisposition to SSc (OR 2.55, 95% CI 1.11 to 5.57,