between healthy and SSc group. In SSc, Spearman analysis showed that anti-CarP inversely correlated with the modified Rodnan skin score (RSS) (R= -0.325, $p<0.001$), independently of patients' age. Receiver operating characteristics (ROC) analysis identified the anti-CarP cutoff that best discriminated dichotomized clinical variables related to skin involvement. This cutoff that was employed to subdivide SSc patients into anti-CarP positive and anti-CarP negative patients. Three SSc skin-related clinical parameters were significantly different between groups: RSS (p=0.001), SI skin (p=0.002), and scleredema ($p<0.001$). A worse skin involvement was associated with low anti-CarP levels.

Conclusions: The study shows that anti-CarP Ab serum level inversely associates to the severity of skin involvement in SSc patients. One possible mechanism to explain the inverse association is that the disease-dependent accumulation of carbamylated proteins in the skin may neutralize circulating anti-CarP Ab, thus contributing to their serum levels decrease. However, further investigation is needed to clarify this issue and to assess whether the levels anti-CarP Ab can be useful in the clinical setting of SSc.

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AB0172 PSGL-1 AND ADAM8 ON DENDRITIC CELLS ARE ASSOCIATED WITH SYSTEMIC SCLEROSIS AND COULD ACT AS BIOMARKERS FOR INTERSTITIAL LUNG DISEASE

J. Silván[1], R. González-Tajuelo[1], E. Vicente-Rabaneda[2], A. Pérez-Frías[1], M. Espartero-Santos[1], E. García-Lorenzo[2], I. González-Alvaro[2], S. Castañeda[2], A. Urzainqui[1]. *[1]Immunology; [2]Rheumatology, Hospital Universitario de la Princesa, Madrid, Spain*

Background: Systemic sclerosis (SSc) is a chronic autoimmune disorder with cutaneous, vascular and immune cells abnormalities that lead to extensive cutaneous and visceral fibrosis with high morbidity and mortality. P-Selectin glycoprotein ligand-1 (PSGL-1) is the major ligand for P-selectin. PSGL-1 mediates the initial contacts with endothelial cells during extravasation to inflamed tissues or homing to several tissues in homeostatic conditions. In addition, PSGL-1/P-Selectin interaction contributes to the homeostasis of the immune system by generating regulatory T cells[1]. Importantly, the metalloprotease ADAM8 interacts with PSGL-1 and proteolytically processes it, what could be a regulatory mechanism to control the expression of PSGL-1[2]. Interestingly, mice lacking PSGL-1 develop a progressive SSc-like syndrome[3].

Objectives: To investigate whether PSGL-1 and ADAM8 expression on leukocytes could be implicated in the pathogenesis of SSc.

Methods: PBLs from 47 SSc patients and 35 healthy donors were analyzed by flow cytometry. The percentage of cells expressing PSGL-1, HLA-DR and ADAM8, as well as the membrane (without cell permeabilization) and total (after cell permeabilization) expression were assessed for each leukocyte subset. For cell permeabilization, cells were incubated for 15 min at room temperature with a fixation/permeabilization solution. Positivity was stablished using isotype control antibodies. Comparisons between groups were analyzed with Student t tests or Mann-Whitney U test. For pairwise multiple comparisons one-way ANOVA with Tukey's post hoc test was applied ($p<0.05$, 95% CI). To analyze the possible contribution of PSGL-1, ADAM8 and HLA-DR to SSc pathogenesis, and to explore whether they could be used as biomarkers for SSc, we studied the influence of these molecules using a multivariate logistic regression model.

Results: SSc patients showed increased expression of HLA-DR in antigen presenting cells (B cells, monocytes and dendritic cells), indicating a higher activation of these cells. PSGL-1 expression in B cells was decreased in SSc patients but increased in monocytes, dendritic cells (DC) and T cells. ADAM8 was increased in B and T lymphocytes, monocytes and DC from SSc patients. Overall, we have identified three variables that are associated with SSc: high percentage of ADAM8-expressing pDC, high PSGL-1 expression in cDC and high HLA-DR expression in CD16+ monocytes. Remarkably, highest PSGL-1 expression on conventional DC (cDC) and high levels of ADAM8 on plasmacytoid DC (pDC) associate with interstitial lung disease (ILD), one of the most severe SSc clinical manifestations, suggesting that PSGL-1 and ADAM8 could be prognostic markers of ILD.

Conclusions: This study highlights that PSGL-1 and ADAM8 expression on DC, monocytes and lymphocytes could be implicated in SSc pathogenesis and particularly on DC might act as biomarkers for ILD.

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AB0173 MYCOBACTERIAL INFECTION IN SYSTEMIC LUPUS ERYTHEMATOSUS: CLINICAL SIGNIFICANCE AND ASSOCIATED FACTORS. DATA FROM THE REGISTRY OF PATIENTS WITH SLE OF THE SPANISH SOCIETY OF RHEUMATOLOGY (RELESSER)

J. Pego-Reigosa[1], V. Campo[2], A. Lois-Iglesias[3], C. Mouriño[2], F. López-Longo[4], M. Galindo[5], J. Calvo[6], J. Ibañez[7], A. Olivé[8], R. Melero[9], A. FdezNebro[10], J. Bernal[11], C. Erausquin[12], E. Tomero[13], L. Horcada[14], E. Uriarte[15], M. Freire[3], I. Rua-Figueroa[12] on behalf of EASSER-RELESSER. *[1]Hospital e IISGS; [2]H, Vigo; [3]H, Coruña; [4]H Gregorio Marañon; [5]H 12Octubre, Madrid; [6]H, Araba; [7]Povisa, Vigo; [8]H, Germans Trias; [9]H, Ourense; [10]H, Carlos Haya; [11]H, Alicante; [12]H, GCanaria; [13]H, la Princesa; [14]H, Navarra; [15]H, Donostia, Spain*

Objectives: To study the prevalence of mycobacterial infection (MI), the associated factors and their clinical significance in patients included in a large SLE cohort.

Methods: Retrospective descriptive study of RELESSER patients with a history of MI and analysis of the factors associated with this infection.

Results: In RELESSER 3,658 patients with ≥4 ACR SLE criteria were included. 90% are women with a mean age of 32.9 years. 93% are Caucasians. The mean follow-up time (± SD) was 120.2 (± 87.6) months. 705 (19.3%) patients had ≥1 severe infection (defined as requiring admission); 1,227 severe infections occurred. MI were diagnosed in 42 patients (1.2% of all RELESSER patients, 3.4% of all severe infections), 85.7% women. The incidence rate of MI was 1 per 1,000 patients/year (95% CI:0.7–1.4).

MI presentation was pulmonary in 18 (42.9%) patients and extrapulmonary in 24 (57.1%) patients [joints in 8 (19.0%) patients, soft tissue in 6 (14.3%) and other sites in 10 (23.8%)]. The extrapulmonary form was associated with immunosuppressants use: 84.6% of the 13 patients treated with immunosuppressive drugs versus 44.4% of the 27 patients without (p=0.01). We did not observe this association with the use of corticosteroids.

To study the factors associated with MI, we performed a bivariate analysis including the variables associated with severe infection in RELESSER (age, sex, ethnicity, corticosteroids, immunosuppressants, antimalarials, previous admission by SLE activity, rituximab and anti-TNF use, Katz severity index, SDI index, SLEDAI index and Charlson comorbidity index). There is a statistically significant association with previous admission by SLE activity (RR:2.9, 95–95%:1.3–6.2, p=0.007), renal impairment (RR:2.0, CI 95%:1,1–3,7, p=0,04), the Katz score (RR:2.1, 95% CI:1.1- 4.0, p=0.04) and the Charlson index (RR: 2.5; 95% CI: 1.3–4.8, p=0.009). Damage (SDI>0) was closely associated with significance:RR: 2.0; 95% CI: 1.0–4.0, p=0.07. Iimmunosuppressants use was associated with an important increase in the risk of MI: RR:4.3; 95% CI:2.2–8.3, p=0.31.

Two patients (4.8%) died (1 respiratory and 1 extrapulmonary). Mean survival after MI diagnosis in these cases was 21 days.

Conclusions: MI in RELESSER affects 1.15% of patients. Its incidence rate is 1 per 1,000 patients/year (95% CI:0.7–1.4). Extrapulmonary localization affects more than half of the patients and is associated with immunosuppressants use. Previous admission by SLE activity, renal involvement, SLE severity and increased number of comorbidities are factors associated with MI.

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AB0174 LYMPHOCYTE SUBSETS T, B AND NK CELS IN SYSTEMIC SCLEROSIS

L. Schneider[1], V. Hax[1], N. Marcondes[2], R.M. Xavier[1], R. Chakr[1]. *[1]Rheumatology; [2]HCPA, Porto Alegre, Brazil*

Background: Systemic sclerosis (SSc) is a rare multisystem disease with underlying immune mechanisms, whose pathogenesis remains unclear. Few previous reports have evaluated lymphocyte subpopulations in SSc and your results are conflicting.

Objectives: The present study aimed to analyze the lymphocyte subsets in SSc patients in comparison to healthy individuals.

Methods: Peripheral blood (PB) samples to analyze lymphocyte subsets were obtained from a non-random convenience sample of 20 SSc patients. Twenty healthy individuals recruited from the blood bank were used as sex and age-matched controls. Blood samples were analyzed by flow cytometry for total T cells, CD4+ and CD8+ T cells subsets, CD19+ B cells and total NK cells. Statistical analyses were performed using the IBM Statistical Package for Social Sciences (SPSS 18.0). Data are expressed as mean ± SD and median and range. Non-parametric Mann–Whitney U test was used for analyses of the flow cytometry. A probability $p<0.05$ was considered statistically significant.

Results: The mean (SD) age of SSc patients was 57.9 (14.2) years, 95% were female and 31.6% presented diffuse cutaneous SSc (dcSSc). Patients presented a lower mean total lymphocyte count compared to healthy controls (23.7% vs.

29.6%, p=0.026) (Table 1.). No statistically significant differences were found in the percentages or the absolute numbers of T, B or NK cells.
Conclusions: Our data support previous reports indicating that depletion of lymphocyte in the PB of SSc patients. However, we found no significant difference in relation to lymphocyte subtypes, which differs from the literature data.
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AB0175 INHIBITORY EFFECT OF ENDOTHELIN-1 TYPE A RECEPTOR ANTAGONISTS ON MIGRATION OF NEUTROPHILS AND TUMOUR CELLS

L.M. Kappes[1], S. Sommerlatte[1], C. Plattfaut[2], S. Pitann[1], G. Marschner[1], H. Heidecke[3], P. Lamprecht[1], A. Müller[1], F. Gieseler[2], O. Cabral-Marques[1], G. Riemekasten[1]. *[1]Department of Rheumatology; [2]Department of Oncology, University of Lübeck, Lübeck; [3]Celltrend GmbH, Luckenwalde, Germany*

Background: Endothelin-1 type A receptor (ETAR) antagonists (e.g. ambrisentan) are currently approved by the U.S Food and Drug administration, representing a well-tolerated treatment of pulmonary arterial hypertension (PAH)[1] for patients with connective tissue diseases such as systemic sclerosis (SSc). Noteworthy, increased numbers of infiltrating neutrophils have been associated with worse clinical outcome in PAH patients. In another context, several studies have reported that endothelin-1 and its receptor ETAR also play a central role in the development of tumour cell invasion and metastasis. However, the effects of ETAR antagonists on migration of neutrophils and tumour cells remain to be determined.
Objectives: The objective was to analyse the effects of two ETAR antagonists on migration of neutrophils and tumour cell lines.
Methods: The migratory ability of peripheral neutrophils from healthy donors (HD) and different tumour cell lines (myeloid leukaemia HL60 cells and human pancreatic adenocarcinoma COLO357 cells) was analysed in response to N-Formylmethionyl-leucyl-phenylalanine (FMLP) or Protease-activated receptor 2 (Par-2) agonist. Because it has been shown before [2], IgGs from HD and SSc patients were used as additional stimulus for migration. Neutrophils and HL60 cells were preincubated (1h) with sitaxentan or ambrisentan, respectively, before being tested for migration (1h) using the Transwell assay. COLO357 cells were incubated (48h) in the presence of sitaxentan and migration was tested in the Oris Pro Cell assay. Migration was analysed by automatic cell counting or digital photo analysis and a migration index was calculated.
Results: Sitaxentan and/or ambrisentan significantly blocked the migration of neutrophils and tumour cell lines. In more detail, neutrophil migration in response to FMLP, being set to 100%, was completely inhibited by sitaxentan (0,46%). Further, neutrophil migration in response to IgGs from HD and SSc patients was induced equally, again being set to 100%. In the presence of sitaxentan migration was reduced to 60%, respectively. In DMSO-differentiated HL60 cells the migratory capacity in response to FMLP (100%) was reduced to 66% by ambrisentan and to 14% by sitaxentan. Moreover, in the presence of sitaxentan and a Par-2 agonist the migratory ability of COLO357 cells was significantly decreased to 89% compared to Par-2 agonist only, being set to 100%.
Conclusions: Our results suggest a pivotal and non-redundant role of ETAR in cell migration, which needs further clarification in order to repurpose the use of ETAR inhibitors. Therapeutic switching of ETAR antagonists from PAH to cancer therapies is a promising adjuvant therapy.
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AB0176 MITOCHONDRIAL DYSFUNCTION IN IDIOPATHIC INFLAMMATORY MYOPATHY DERIVED MYOBLASTS

C. Basualto-Alarcón[1], M.F. Bozán[2], J. Bevilacqua[1,2], F. Urra[1], D. González[1], H. Gatica[2], A. Göecke[2], P. Wurmann[2], M.F. Sabugo[2], S. Saavedra[2], C. Cárdenas[1,3,4]. *[1]Cellular Metabolism and Bioenergetics Laboratory; Anatomy and Developmental Biology Program; ICBM; Faculty of Medicine., Universidad de Chile; [2]Hospital Clínico Universidad de Chile; [3]Geroscience Center for Brain Health and Metabolism, Universidad de Chile, Santiago, Chile; [4]Buck Institute for Research on Aging, Novato, United States*

Background: Idiopathic inflammatory myopathies (IIM) are acquired skeletal muscle diseases, characterized by proximal muscle weakness. This syndrome includes five different (1) diseases, nevertheless, in this work, we included dermatomyositis (DM) and polymyositis (PM) patients. Has been reported that despite treating inflammation, muscle atrophy and weakness persist in some patients, suggesting an inherent muscle cause (2,3). In addition, histological studies show some mitochondrial abnormalities. Our aim was to evaluate a possible role for mitochondrial dysfunction in the pathophysiology of this diseases.
Objectives: To compare the mitochondrial status of myoblast obtained from myositis patients -and healthy control- biopsies.
Methods: Primary cultured myoblasts extracted from the deltoid of IIM patients were used and compared with myoblasts obtained from normal patients subjected to shoulder surgery. Also, a human skeletal muscle cell line (RCMH) was used as control. The bioenergetic profile was analyzed with an Extracellular flux analyzer^{e}96 (Seahorse Biosciences). Also, biopsy tissue was used for Western blot (WB) and immunofluorescence experiments.
Results: Basal oxygen consumption rate, ATP-linked oxygen consumption, maximal oxygen consumption and spare respiratory capacity were lower in IIM myoblasts when compared to RCMH myoblasts, however, when compare to control primary cultured myoblasts we only find differences in ATP-linked oxygen consumption. Expression levels of mitochondrial complexes (I, III, IV and V) were analyzed by WB in tissue samples. No differences were observed between control and IIM patients. Mitochondrial area was estimated by immunofluorescence of the voltage dependent anion channel (VDAC), which show no differences between control and patients.
Conclusions: IIM derived myoblasts present a compromised mitochondrial function, compared to control myoblasts. Specially, oxygen consumption associated with ATP synthesis show decreased levels in patients. Although, expression levels of the mitochondrial complexes as well as mitochondrial area were not different between control and patients. Future experiments should address if IIM mitochondria are consuming less oxygen because of a lower ATP demand or because a primary mitochondrial damage.
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AB0177 INCREASED LEVELS OF THE INFLAMMATORY PROTEINS CXCL10, CXCL11, TNFR2 AND YLK-40 TYPIFY THE EARLIEST PHASE OF SYSTEMIC SCLEROSIS (SSC)

M. Cossu[1,2], L. van Bon[1], M. Rossato[1,2], L. Beretta[3], T.R.D.J. Radstake[1,2]. *[1]Rheumatology & Clinical Immunology; [2]Laboratory of Translational Immunology (LTI), UMC Utrecht, Utrecht, Netherlands; [3]Referral Center for Systemic Autoimmune Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*

Background: Definite systemic sclerosis (defSSc) patients yet lacking the prototypical signs of fibrosis stratify in an intermediate severity stage between pre-clinical and fibrotic, limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) phenotypical subsets[1].
Objectives: We aimed to molecularly position defSSc patients in respect to healthy controls (HC) and early SSc (EaSSc) patients with a broad panel of serum mediators of inflammation and tissue damage in an attempt to increase the knowledge in pathophysiologic mechanisms and biomarkers availability, thereby offering new ground for disease interception before fibrosis develops.
Methods: To this end, an 88-plex immunoassay was performed in sera from an identification cohort composed of 21 EaSSc according to LeRoy and Medsger criteria[2] without other signs and symptoms of evolutive disease, 15 defSSc patients according to the 2013 ACR/EULAR criteria[3] without skin or lung fibrosis and 11 HC. A larger cohort comprising 47 EaSSc, 48 defSSc and 43 HC was used for replication purposes. Fifty-one lcSSc and 35 dcSSc as comparison with established, fibrotic disease were recruited in parallel.
Results: Sixteen mediators differentially expressed in EaSSc and defSSc were selected for replication (one-way ANOVA and/or ANOVA polynomial test for trend with exploratory threshold $p<0.1$). Amongst these, after correction for multiple comparisons, CXCL10/IP-10, CXCL11/I-TAC, TNFR2 and CHI3L1/YKL-40 showed a significant upregulation in defSSc and EaSSc with a linear increase from HC to EaSSc to defSSc. The level of upregulation observed in defSSc individuals was similar (CXCL10/IP-10, CXCL11/I-TAC) or further increased (TNFR2, CHI3L1/YKL-40) in lcSSc and dcSSc patients. A set of 7 ranked markers (Angiopoietin-2, TNFR2, CXCL11/I-TAC, CXCL10/IP-10, sICAM-1, CHI3L1/YKL-40, CXCL9/MIG) provided a good visualization of the gradually increasing pattern from HC to EaSSc to defSSc to lcSSc and dcSSc.
Conclusions: This is the first attempt to validate circulating biomarkers defining the earliest phases of SSc. Despite the need for confirmation in a prospective