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FRI0365 NEW COLLAGEN BIOMARKERS PREDICT PROGRESSION OF FIBROSIS IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is a complex autoimmune disease with extensive fibrosis of the skin and internal organs in which extracellular matrix (ECM) remodeling is a key pathogenic process. Imbalance in the formation and degradation of collagens results in fibrosis. Quantifying the tissue turnover in a highly fibrotic disease such as SSc is very important for the prediction of disease progression and therapeutic efficacy. Given the clinical heterogeneity of SSc patients, biomarkers facilitating personalized medicine approaches are highly needed.
Objectives: To evaluate the potential of selected ECM neo-epitopes as serological biomarkers for diagnosis, prediction of clinical outcomes and disease progression in SSc.
Methods: Healthy controls (HC; n=29), stable SSc (n=149), and progressive SSc patients (n=23, progression defined either as 10% decrease in FVC% predicted or increase in mRSS ≥25% and 5 points on one year clinical follow up), meeting the 2013 ACR/EULAR classification criteria were analyzed. Longitudinal clinical assessment, data recording and sera collection were done according to EUSTAR standards. ECM-degradation (C3M, VICM, C4M2, BGM) and ECM-formation biomarkers (P1NP, P4NP7S, Pro-C3, Pro-C5, Pro-C6) were measured in serum using newly developed ELISA-based assays (Nordic Bioscience). Differences in biomarker levels were analyzed with respect to several fibrosis-related clinical outcomes. Statistical analysis was performed by Man-Whitney U, Kruskal-Wallis and Spearman tests. Biomarkers' sensitivity and specificity was examined by ROC analysis.
Results: Both ECM-degradation and ECM-formation biomarkers differed between SSc patients and HC: The expression of C4M2, Pro-C3, BGM and C3M was significantly increased in SSc patients compared to HC ($p<0.0001$, AUC=0.93; $p<0.0001$, AUC=0.74; $p=0.003$, AUC=0.67; $p<0.0001$; AUC=0.94, respectively), whereas P1NP was significantly lower ($p<0.0001$, AUC=0.78), Figure 1. Furthermore, Pro-C3, VICM and Pro-C6 levels were significantly higher in SSc progressors vs. HC ($p<0.0001$, AUC=0.86; $p=0.003$, AUC=0.75; $p=0.0005$, AUC=0.81, respectively).
Currently, there is no fully validated biomarker predicting worsening of fibrosis. In this regard, most interestingly, the ECM-degradation markers C4M2, BGM, C3M were significantly lower in SSc patients showing progression of fibrosis on follow up versus SSc patients being stable on follow up ($p<0.0001$; $p<0.008$; $p<0.0001$, respectively). Consistently, the formation marker Pro-C6 was significantly increased ($p=0.001$, AUC=0.71) indicating a profound imbalance of ECM turnover in progressors. The strongest difference between progressive and stable SSc patients was seen for the ratio between the formation and degradation biomarkers Pro-C3/C3M which showed an AUC of 0.86 in progressive vs. stable SSc patients ($p<0.0001$).
Conclusions: These data support ECM neo-epitopes as potential new biomarkers of prognostic interest in SSc. This could help identifying patients at risk of progression of their fibrotic disease at one year follow up. The significant decrease in ECM-degradation markers in SSc progressors compared to stable patients suggests an impairment of collagen degradation in this group. The ratio of Pro-C3/C3M is as a new potential predictive index for differentiation of stable vs. progressive patients.
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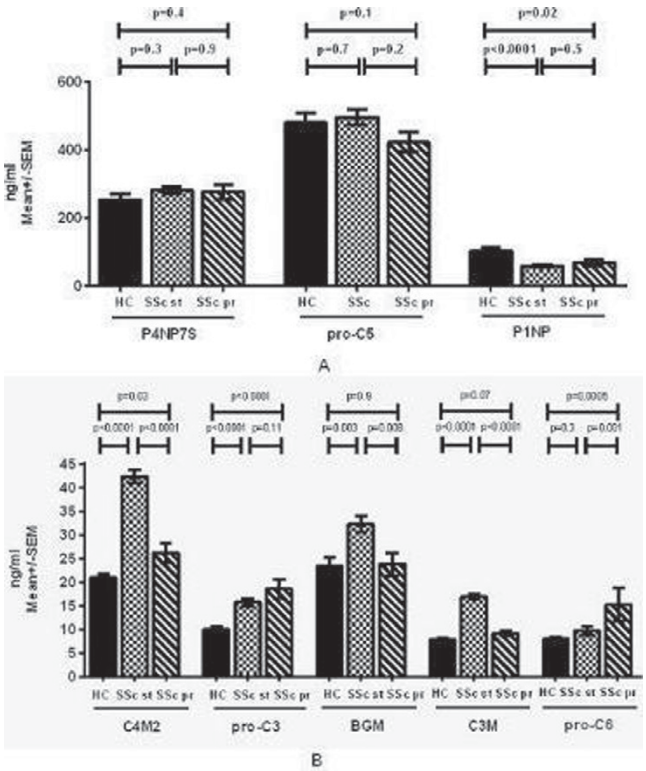

Figure 1: Expression of biomarkers in healthy controls (HC), stable SSc patients (SSc) and progressive SSc patients (SSc pr). A) P4NP7S- amino terminal pro-peptide of procollagen type IV; Pro-C5- formation of collagen type V; P1NP-amino terminal pro-peptide of procollagen type I; B) C4M2-MMP degraded collagen type IV ; Pro-C3- formation of collagen type III ; BGM -biglycan degraded by MMP-2/9; C3M-collagen III degraded by MMP-9; Pro-C6- formation of collagen type VI;

FRI0366 A PILOT STUDY ON ISCHEMIA AND REPERFUSION INJURY DURING A RAYNAUD'S ATTACK: SEQUENTIAL ASSESSMENT OF REDOX STRESS PARAMETERS IN A UNIQUE COOLING AND REWARMING EXPERIMENT

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Background: Oxidative stress plays a role in systemic sclerosis (SSc), but the molecular mechanisms involved are incompletely understood. During an attack of Raynaud's phenomenon (RP) a period of ischemia (I), followed by reperfusion (R) occurs frequently, associated with the severity of vasculopathy. [1] Only in secondary RP digital ulcers develop. We hypothesized that I/R injury may play a role in the pathogenesis and could offer new therapeutic targets.
Objectives: To explore the course of oxidative stress in patients with SSc compared with primary RP and healthy controls.
Methods: A total of 30 patients were included: 10 with limited cutaneous SSc (age: 57 (53–61) yr, male/female 5/5), 10 with primary RP (age: 54 (41–58), 2/8), and 10 healthy controls (age: 25 (22–25), 3/7). A standardized cooling experiment was performed and digital perfusion was assessed in all 5 fingers using photo-electric plethysmography: at baseline (T=0) the dominant hand was submerged in water at 33℃, followed by cooling in steps of 3℃ every 4 minutes, until 6℃ or when pain became intolerable (T=1). Recording was continued 10 (T=2) and 30 (T=3) minutes of rewarming to ambient temperature (23℃). Blood was drawn from ipsilateral cubital vein at T0, 1, 2 and 3, markers for tissue injury (lactate, LDH, creatinine phosphokinase (CPK) [routine methods]), redox status (free thiols corrected for total protein) and nitric oxide (NO) activity (NO_2^-, NO_3^-, RXNO) were measured in plasma. [1–3] Numbers are in median (IQR).
Results: Baseline free thiols were significantly decreased in RP vs. controls (5.18 (4.79–5.63) vs 5.87 (5.41–5.99) umol/g, p=0.013), with no differences in lactate, LDH, CPK, and NO activity. Raynaud's attack was induced in all RP patients but not in controls. Median duration of hypoperfusion was greater in SSc vs. PRP (30 (27–35) vs. 12 (9–14) min, p=0.010), with a considerably longer recovery time (8 (4–10) vs. 0 (0–1) min, p=0.006). No changes were observed in lactate, LDH, and CPK levels. A rise in free thiols occurred at recovery (T4) in all 3 groups (figure 1). The concentrations of NO-related products did not change during cooling or recovery. No association was detected between the extent of I/R and plasma parameters.
Conclusions: In patients with RP free thiols were significantly reduced, indicating increased redox stress. During the cooling and rewarming experiment, a clear rise in free thiols was observed during rewarming, irrespective of the underlying disease or finger perfusion. Meanwhile, NO-related products remained stable.

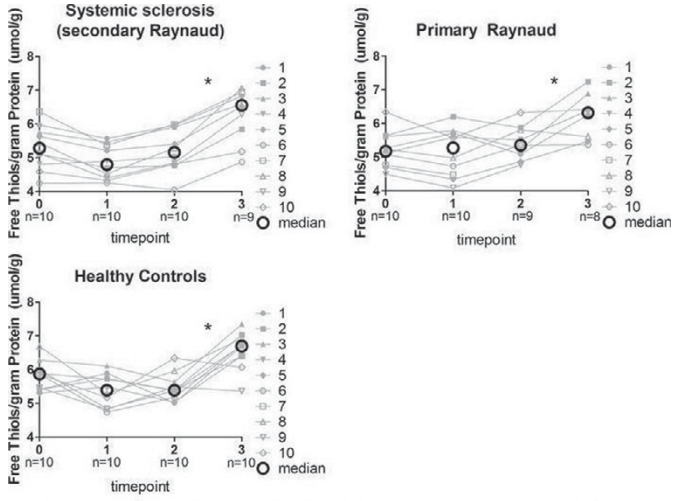

Figure 1. Free thiols per gram protein in all groups * p<0.005 between timepoint 2 and 3

Although these findings need further study, they may suggest activation of ubiquitous antioxidant defense mechanisms during cooling and/or rewarming and should be explored for future use as a potential therapeutic target in RP.

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FRI0367 NEW AUTOIMMUNE TARGETS IN IDIOPATHIC INFLAMMATORY MYOPATHIES - AN ANTIGEN BEAD ARRAY APPROACH

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Background: The Idiopathic Inflammatory Myopathies (IIM) is a group of rare systemic inflammatory diseases characterized by severe organ involvement and premature mortality. Several myositis specific auto-antibodies (MSAs) have been recognized and associated with specific clinical manifestations and prognosis, still many patients are autoantibody negative. Identification of new autoimmune targets will be helpful in improving diagnosis, better stratifying into subgroups, prediction of prognosis, tailoring treatment and to understand underlying biological pathways.
Objectives: To identify new autoimmune targets in IIM by antigen bead array (1).
Methods: A bead array with 354 antigens was used to explore the autoimmune reactivity in 881 plasma samples from patients with IIM (N=225), Systemic Lupus Erythematosus (SLE) (N=350) and population controls (N=306). The antigens were selected from initial screenings of 160 SLE-samples on a total of 5760 antigens on planar arrays, and a first verification bead array with 355 antigens. The IIM samples represented three groups of patients with distinct diagnoses: Dermatomyositis (DM, N=83), Polymyositis (PM, N=111) and Inclusion Body Myositis (IBM, N=31), who were regularly followed at the Rheumatology Unit of the Karolinska University Hospital from January 2003 until March 2014. Based on 2 possible levels of cutoff, each sample was classified as reactive to each single antigen (Ag) at low or high cut off or non-reactive.
Results: In general, depending on the cutoff stringency, 86–88% of the 354 selected antigens showed reactivity in at least one sample with no difference between IIM, SLE and controls. Comparing PM, DM, IBM according to the number of samples which showed reactivity towards each single Ag, reactivity at high cut off towards NADH dehydrogenase 1 α subcomplex 11 (NDUFA11), poly(A) RNA polymerase D4 (PAPD4), CD163, l(3)mbt-like 1 (L3MBTL1) and calcium release-activated calcium modulator 2 (ORAI2) was discovered with higher frequencies in the IBM samples compared to PM and DM. In the group of IIM patients testing negative for all the known MSAs increased reactivity at high cut off was observed towards E3 ubiquitin protein ligase 2 (SIAH2), leiomodin 2 (LMO2) and RAD23 homolog A (RAD23A). In the group of IIM patients with history of malignancy and no evidence for anti-p155/140 antibodies the antigens early B-cell factor 2 (EBF2), POU class 6 homebox 1 (POUF61) and growth differentiation factor 7 (GDF7) revealed high serum reactivity. In IIM patients with interstitial lung disease increased reactivity at high cut off was found towards zinc finger protein 688 (ZNF688) and prostaglandin D2 receptor (PTGDR). A high frequency of known target reactivities (MSAs) was also confirmed.
Conclusions: Reactivity towards autoantigens corresponding to human proteins was present in plasma samples from IIM, controls, and SLE. Potentially new autoimmune targets have been discovered in IIM subgroups, although further validation in independent cohorts is needed.

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FRI0368 NAILFOLD CAPILLAROSCOPY CHANGES REFLECT ENDOTHELIAL ACTIVATION AND INJURY IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is a severe connective tissue disease characterized by vascular and fibrotic changes in the skin and various internal organs. Pathogenesis of SSc includes early-onset vasculopathy with endothelial cell activation, microvascular injury and impaired angiogenesis.
Objectives: We aimed to determine the association of several biological molecules reflecting endothelial cell activation or dysfunction: E- selectin (E-sel), inter-cellular adhesion molecule 1 (ICAM-1), endothelin 1 (ET-1), von Willebrand factor (vWF) and interleukin 6 (IL-6), with distinct capillaroscopic SSc patterns and with more severe disease.
Methods: Forty consecutive SSc patients attending our EUSTAR SSc clinic, aged [median (IQR)] 52 (18) years, male gender 4/40 (10%), diffuse cutaneous subset (dcSSc) 14/40 (35%) were enrolled in this study. Extensive clinical and nailfold capillaroscopy (NFC) pattern assessment, as well as quantification of serum E-sel, ICAM-1, ET-1, vWF, IL-6 and C-reactive protein (CRP) were performed on all patients. Associations between vascular biomarkers and disease characteristics were evaluated by Mann-Whitney U-test and Spearman correlations.
Results: NFC "late" pattern was found in 21 patients, while 6 had "early" and 13 had "active" NFC pattern. All 5 vascular biomarkers correlated with each other good to moderately, with r indices varying between 0.660 and 0.332, the only exception being ET-1 which did not correlate with E-sel. Good correlations (r 0.465 to 0.727) were also found between all 5 biomarkers and CRP. Patients with severe vasculopathy, as reflected by the NFC "late" pattern, had higher levels of IL-6 (median 12.06 vs. 3.08 pg/mL, p=0.001), ET-1 (median 2.06 vs 1.59 pg/mL, p=0.029), vWF (median 3284 vs 2730 IU/mL, p=0.013) and E-sel (median 52.6 vs. 42.3 ng/mL, $p>0.05$), compared to patients with NFC "early" or "active" patterns. There was a significant, negative correlation between lung transfer for carbon monoxide (DLCO) and E-sel, ICAM-1 (both $p<0.001$) and vWF (p=0.013). ET-1 was higher in patients with more severe disease (dcSSc, patients positive for anti-topoisomerase antibodies and patients with a history of digital ulcers – all $p<0.05$).
Conclusions: Serum biomarkers reflecting endothelial cell activation and/or dysfunction are elevated in patients with more severe SSc-associated vasculopathy and correlate with serum CRP. Together with NFC data they might be used for assessing vasculopathy severity in SSc and identifying patients who would benefit from more aggressive vasoactive treatment.
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FRI0369 PROSPECTIVE EVALUATION OF THE CAPILLAROSCOPIC SKIN ULCER INDEX (CSURI) IN CLINICAL PRACTICE

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Background: Nailfold videocapillaroscopy (NVC) is an imaging technique representing a reliable tool for the classification, diagnosis and monitoring of systemic sclerosis (SSc) patients. The capillaroscopic skin ulcer index (CSURI) was suggested to identify patients at risk of developing digital ulcers (DU) [1].
Objectives: This study aims (1) to describe the practicality of the CSURI in clinical practice, (2) to describe the change of CSURI during follow-up, and (3) to assess associations between the change in CSURI and demographic and disease characteristics.