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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 $\geq$ 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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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°C, followed by cooling in steps of 3°C every 4 minutes, until 6°C or when pain became intolerable (T=1). Recording was continued 10 (T=2) and 30 (T=3) minutes of rewarming to ambient temperature (23°C). 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 (NO₂⁻, NO₃⁻, 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) μ mol/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: 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-bigglycan degraded by MMP-2/9; C3M-collagen III degraded by MMP-9; Pro-C6-formation of collagen type VI.