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POS0424   **DETERMINING CIRCULATING ENDOTHELIAL CELLS USING CELLSEARCH SYSTEM IN SYSTEMIC SCLEROSIS PATIENTS**

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Background: Endothelial damage and fibroproliferative vasculopathy of small vessels are pathological hallmarks of Systemic Sclerosis (SSc). Detection and analysis of circulating endothelial cells (CECs) detached from affected blood vessels may be an informative tool to study vascular dysfunction and could be considered a novel biomarker of scleroderma vasculopathy. Our group first showed the presence of CECs in SSc by fluorescence-activated cell sorting (FACS), demonstrating that a raised counts of active CECs may represent direct evidence of active vascular disease in SSc. Despite these interesting data, issues related to difficulties in CEC counting through FACS analysis, due their very low concentration in peripheral blood, prevented further investigations in this field. Recently, a specific kit for the detection of CECs has been developed through the CellSearch System (CS), a semi-automated device for the standardized analysis of rare cells, such as CECs, in peripheral blood.
Objectives: To assess the counts of CECs determined by the CS in SSc patients and to evaluate their clinical implication and potential as vascular biomarker in SSc.
Methods: 10mL of blood samples were collected from 29 subjects (19 SSc patients and 10 healthy donors - HDs) and stored in tubes containing a specific preservative, to allow the analysis of 4mL of blood within 72 hours, according to manufacturer instructions. Out of 19 SSc patients, 18 were female, 10 had the limited form and 9 the diffuse cutaneous variant of SSc. CS uses a proprietary kit containing a ferrofluid-based reagent, that target CD146 to magnetically capture CECs, and the immunofluorescent reagents to stain the CECs, defined as CD146+, CD105-PE+, DAPI+ and CD45-APC-. Clinical, laboratoristic and demographic data were also collected.
Results: The mean number of CECs in patients with SSc was significantly higher in comparison to HDs (554/4mL vs. 53.5/4 mL, p=0.0042). When analyzed according to disease subset, both lcSSc and dcSSc showed significantly increased levels of CECs in comparison with HDs (p=0.003 and p=0.005, respectively). No statistical difference was observed in the mean number of CECs in patients with lcSSc compared to those with dcSSc. Regarding vascular involvement, the CECs counts strictly correlated with the presence of digital ulcers (DUs) (p=0.0001) showing a median of 863cells/4mL for the SSc patients with DUs versus a median of 276.2/4mL for the SSc patients without DUs. No statistical correlation was found between CECs and serological autoantibody pattern, skin parameters, or joint and muscle involvement. Patients with active disease, according to the EUSTAR Activity Index, showed a higher CECs value than those with inactive disease (p=0.0012).
Conclusion: The amount of CECs detectable in peripheral blood has been recently proposed as a marker of endothelial damage in different vascular diseases, including SSc. However, currently no standardized method is available to determine CEC counts, which makes reported data on CECs reliable and suitable. The CS system is a commercially available semi-automated system that enables standardized determination of CECs. Thus, we examined clinical utility of CECs count by this system in SSc patients. Our results confirm that baseline CEC counts, evaluated by a new standardized method, may represent direct evidence of endothelial damage in SSc and could be a promising tool for monitoring active disease and evaluating therapeutic responses to vascular and immunosuppressive treatments.

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POS0425   **INCREASED KERATIN 9 EXPRESSION IN SYSTEMIC SCLEROSIS SKIN IS DRIVEN BY THE LNCRNA HOTAIR FROM FIBROBLAST DERIVED EXOSOMES**

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Background: Skin fibrosis is the hallmark fibrotic manifestation of systemic sclerosis (SSc). Despite a key role of tissue fibroblasts, skin changes extend to the keratinocyte layer, which contribute to the loss of skin function. RNA seq. analysis of SSc patient forearm skin showed that palmoplantar specific Keratin 9 (CK9) was highly expressed (1). SSc affected skin shares several features with palmoplantar skin including increased keratinocyte layer thickness and lack of hair. Seminal work of last decade has shown that long noncoding RNA in the HOX loci play a crucial role in skin keratinocyte differentiation (2), with the lncRNA HOTAIR being one of the HOX lncRNA mostly expressed in the palmoplantar region.
Objectives: Following recent data suggesting a role of HOTAIR in the profibrotic phenotype of dermal fibroblasts in SSc (3), here we set out to determine if HOTAIR expressed in SSc dermal fibroblasts was a contributing factor to the high levels of CK9 found in SSc patient skin
Methods: Full-thickness skin biopsies were surgically obtained from the forearms of patients with SSc of recent onset. Fibroblasts were isolated and cultured in monolayers. HOX transcript antisense RNA (HOTAIR) was expressed in healthy dermal fibroblasts by lentiviral induction employing a vector containing the specific sequence. Exosomes were isolated from dermal fibroblast media using the Total exosome isolation reagent (Thermo Fisher). Enhancer of zeste 2 (EZH2) was blocked with GSK126 inhibitor. Skin equivalents were created using scramble and HOTAIR expressing fibroblasts with primary keratinocytes.
Results: Media from both SSc patient fibroblasts and HOTAIR expressing fibroblasts induced CK9 expression in healthy keratinocytes *in vitro*. In addition, HOTAIR expressing fibroblasts induces CK9 expression in keratinocytes in 3D skin equivalent models. Media fractionation studies indicated that HOTAIR was present in fibroblasts exosomes and found at a higher concentration (2.7 fold p=0.01) in exosomes from SSc fibroblasts. Importantly, transfection of Exosomal RNAs from SSc fibroblasts could reproduce the increase in CK9 in keratinocytes. Mechanistically, CK9 induction was mediated by changes to the histone methylation profile in the keratinocytes through EZH2.
Conclusion: Pro-fibrotic dermal fibroblasts in systemic sclerosis contribute to the overall skin loss of function by inducing CK9 in adjacent keratinocytes through transfer of the long non-coding RNA HOTAIR. Unraveling the crosstalk of activated fibroblasts with adjacent cells may lead to identify therapeutic targets to re-establish tissue homeostasis and function during fibrosis.

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POS0426   **CIRCULATING MICRORNA PROFILING IN PATIENTS WITH ANTI-SYNTHETASE SYNDROME AND INTERSTITIAL LUNG DISEASE**

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Background: Anti-synthetase syndrome (ASSD) is an autoimmune disease characterized by autoantibodies against one of many aminoacyl transfer RNA (tRNA) synthetases. Interstitial Lung Disease (ILD) in ASSD patients is frequent, often severe and rapidly progressive, causing much of the increased morbidity and mortality associated with ASSD as compared to other idiopathic inflammatory myopathies [1].