311. MULTIPLEX CYTOKINE ANALYSIS OF DERMAL INTERSTITIAL BLISTER FLUID IN SYSTEMIC SCLEROSIS DEFINES POTENTIAL PATHOGENIC PATHWAYS AND DIFFERENTIATES CLINICAL SUBSETS

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Background: Clinical diversity in SSCs likely to reflect multifaceted pathogenesis and the effect of key growth factors or cytokines operating within a disease-specific microenvironment. Dermal interstitial fluid sampling offers the potential to examine local biological mechanisms and define protein expression within lesional tissue. We have used multiplex cytokine analysis to define the inflammatory and immune activity in the lesions of SSC patients.

Methods: Interstitial fluid samples from the forearm skin of patients (n = 25; DcSSc = 19; LcSSc = 6) or healthy controls (HC) (n = 10) were collected using a dermal suction blister method, together with contemporaneous serum samples. Both the serum and interstitial fluid samples were profiled by Luminex array for inflammatory cytokines, chemokines, and growth factors. Permutation analysis was used to compare cytokine levels in SSC and HC samples, as well as to compare the difference between serum samples and healthy control samples. Pearson’s correlation coefficient was used to assess
any correlation between serum and interstitial fluid samples within the patient samples.

**Results:** Luminex array profiling of the dermal blister fluid showed increased inflammatory cytokines (mean IL-6 in SSc-77.2 pg/ml, HC-17.8 pg/ml, P = 0.009, mean IL-17 in SSc-0.61 pg/ml, HC-0 pg/ml, P = 0.03), and vascular growth factors (VEGF21.7 pg/ml in SSc, HC-13.5 pg/ml (P = 0.26), PDGF-aa 16.4 pg/ml in SSc, HC-0.97 pg/ml, P = 0.049). Additionally, MCP-3(CCL7), IL-15 and IFN-g were all significantly increased in SSc compared with HC (P < 0.05). Within the serum, Luminex array profiling highlighted a significant increase in mean IL-12p40 (SSc-31.7 pg/ml, HC-2.05 pg/ml, P = 0.012) and mean IL-1-α (SSc-21.15 pg/ml, HC-2.25 pg/ml, P = 0.029) in SSc compared with controls. There was no significant difference for other cytokines. Comparing the luminex array profiling of the dermal blister fluid from those with SSc, with the paired serum samples, the only significant correlation was in MCP-3 (r² = 0.31, P = 0.013). Other proinflammatory cytokines (IL-6 (r² = 0.083, P = 0.23), IL-17 (r² = 0.02, P = 0.58)) and growth factors (VEGF (r² = 0.03, P = 0.47), PDGF (r² = 0.08, P = 0.22)) showed no correlation between serum samples and dermal blister fluid. Interestingly, the healthy controls showed greater correlation between the Luminex array results from the dermal blister fluid and serum samples, with many reaching significance (IL-10, MCP-3, IL1ra, FGF-2).

**Conclusion:** Our results confirm the potential utility of dermal blister fluid to define local biological processes in SSc, and identifies profibrotic, angiogenic and T-cell derived factors expressed locally within the skin lesions. In contrast, analysis plasma samples revealed elevation of monocyte derived inflammatory proteins. Absence of correlation between the interstitial fluid samples and paired serum samples suggests that the dermal blister sampling method reflects local dermal protein expression rather than exudates from the plasma into the skin. This dermal suction method offers an opportunity to profile the local inflammatory process occurring within the skin and has potential to complement clinical and gene expression based classification to facilitate targeted therapy, as well as providing potential markers of disease activity or treatment effect.

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