compare abundances of cell clusters, the association model Prediction Analysis for Microarrays (PAMR) was applied with a minimum cluster size of 2.2%, 5 cross-validation folds and a false discovery rate of 1%.

Results: Conventional biaxial gating showed that there were large differences in the proportions of both IFN γ ⁺ CD4⁺ T cells (1–15%) and IL-17A⁺ CD4⁺ T cells (0–9%) within and between RA and HC. The proportion of IFN γ ⁺ CD4⁺ T cells (Th1) did not correlate with that of IL-17A⁺ T cells (Th17), leading to the generation of 4 immunophenotypes: “Th1”, “Th17”, “double-hi”, and “low”, determined by cytokine expression.

Using CITRUS we identified 3 clusters of cells which differed significantly in abundance between HC and RA. Cluster 1 was CD4⁺CD38⁺, had characteristics of regulatory T cells and was more abundant in HC. Cluster 2 was CD28^{low}CD8⁺ expressing perforin and Tbet, and cluster 3 a CD127^{high}CCR6⁺ population, both of which were more abundant in RA.

Conclusions: Cytokine expression of *ex vivo* stimulated T cells from RA and HC is highly variable and can be detected using mass cytometry. Cytokine signatures of this kind may be informative when predicting treatment response.

CITRUS identified 3 cell clusters which may have been missed using conventional methods of analysis. Importantly, CITRUS allowed inspection of the phenotype of each cluster.

Our next step is to compare RA responders to non-responders using the methods described. The function of these clusters will be further investigated by cell isolation with fluorescence-activated cell sorting (FACS) and may go some way in predicting treatment response. Finally we recommend the use of both automated clustering algorithms alongside conventional gating methods when analysing high-dimensional data.

Disclosure of Interest: None declared

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OP0095 AT-RISK OF CONNECTIVE TISSUE DISEASE IN AN AT-RISK COHORT USING A NOVEL INTERFERON STIMULATED GENE EXPRESSION SCORE

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Background: A period of ANA positivity and other immune dysregulation precedes connective tissue disease, providing a potential opportunity for disease prevention. Type I interferons (IFN-I) play a role in pathogenesis but their role in disease initiation is unclear.

Objectives: To develop biomarkers of progression to systemic autoimmunity, with a view to enabling early intervention for disease prevention.

Methods: A prospective observational study was conducted in 125 patients At Risk of CTD defined by (i) ANA; (ii) ≤ 1 clinical SLE criteria; (iii) symptom duration < 12 months and (iv) treatment-naïve. Progression was defined by meeting 2012 ACR/SLICC SLE, 2016 ACR/EULAR Primary Sjogren's, or other diagnostic criteria. Expression of 30 selected “selected” ISGs was measured using Taqman. Factor analysis was used to reduce the gene expression data to a limited set of factors, which were compared between patient groups using Mann Whitney U Test. Two factor scores explained 80% of the data variance: “Score A” (composed of IFN- α responsive genes) and “Score B” (genes responsive to IFN- α and γ). 95 healthy controls and 107 SLE patients were used as negative and positive controls.

Results: 82 patients with 1-year follow-up data were studied. 71 were female with mean age 48 ± 15 years. 16 (20%) patients progressed to CTD (SLE=12, Sjogren's=4) in the following 12 months. At baseline, only IFN Score A was increased in both At Risk-CTD and SLE vs healthy controls; $p < 0.001$. IFN Score B was only increased in true established SLE.

In At-Risk patients, IFN Score B was low in patients who did not progress and increased in those who did progress; $p = 0.004$. However, there was no difference in IFN Score A between these two groups; $p = 0.252$ (Figure 1). Although complement levels and lymphocyte counts were lower in SLE, these were not different between the At-Risk progression and non-progression groups. Anti-dsDNA titres were higher in SLE but not different between the progression groups; all $p > 0.10$.

Conclusions: A novel ISG score predicts progression from ANA+ to clinical

autoimmune disease. This may allow early intervention to prevent CTD. Analysis of other clinical, immunological and imaging biomarkers are in progress as well as a validation cohort.

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OP0096 INTERFERON SIGNATURE IN SYSTEMIC SCLEROSIS LUNG MICROVASCULAR ENDOTHELIAL CELLS

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Background: Systemic Sclerosis (SSc) is characterized by severe fibroproliferative vasculopathy, exaggerated deposition of extracellular matrix molecules (ECM) in skin and multiple internal organs, and alterations of humoral, cellular and innate immunity. Vascular changes are responsible for the earliest and most severe SSc clinical manifestations, however, the mechanisms responsible have not been elucidated.

Objectives: The goal of this study was to analyze the gene expression differences between normal and SSc lung microvascular endothelial cells (EC) to improve the understanding of SSc vasculopathy pathophysiology.

Methods: Pulmonary microvascular EC were isolated employing immunomagnetic procedures from lungs from patients with SSc undergoing lung transplantation. Control pulmonary microvascular EC were isolated from autopsies of individuals who died from non-pulmonary causes. Following isolation, microarrays were performed in EC from each group. Expression of genes with the highest differential expression was validated with RT-PCR and Western blots.

Results: Interferon-stimulated genes (ISGs) including IFI44L, IFI44, IFI6, IFIH1, IFIT1 displayed the highest differential expression; being overexpressed in EC obtained from the three SSc donors (Figure 1). Other genes such as those encoding ECM production related proteins, genes associated with post-translational methylation, and genes for numerous chemokines and cytokines were also differentially overexpressed in SSc EC. Increased gene expression and increased protein levels of selected ISGs were confirmed by Western blots.

Figure 1. A: Volcano Plot showing differentially expressed transcripts with 2 fold or greater difference ($p < 0.05$) in expression between SSc and normal control microvascular ECs (130 transcripts corresponding to 119 genes). Selected ISGs are circled (IFI-6, IFIT1, IFI-44, IFI44L, IFIT-3, OAS-1). B: Detail of the resulting heat map of a dendrogram (hierarchically clustered) reveals groups of genes with high expression levels (red squares), low expression levels (blue squares) or background expression levels (yellow squares). Interferon related genes are marked with a red star.

Conclusions: Numerous ISGs are differentially overexpressed in SSc pulmonary microvascular EC in comparison with normal control EC. These results suggest that events leading to an interferon response in these cells may play a role in the pathogenesis of SSc lung vasculopathy.

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