Multiparametric Detection of Autoantibodies to investigate Relationships between Serological and Clinical Subsets of Systemic Sclerosis Patients

Petra Budde[1], Hans-Dieter Zucht[1], Daniel Wirtz[1], Matthias Schneider[2], Peter Schulz-Knappe[1], Nicolas Hunzelmann[3]
[1]Protagen AG, Dortmund, [2]Rheumatology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, [3]Department of Dermatology, University of Cologne, Cologne, Germany

EULAR 2017 SAT0319

Introduction

- Systemic sclerosis (SSc) is a remarkably heterogeneous autoimmune
- disease, for which effective disease-modifying therapies are still lacking.
- The most widely used classification divides SSc into the two major subsets diffuse cutaneous (dcSSc) and limited (lcSSc) SSc by the extent and severity of skin fibrosis. However, not all patients fit into these subsets.
- We explored whether autoantibodies (AAB) against a multitude of antigens could provide biomarkers to uncover unrecognized SSc subtypes and insights into the pathogenesis of SSc.
- Here, we describe the development of a 20 marker multiplexed AAB assay and explored its utility for SSc patient subgroup analysis.

Methods

- Novel SSc-associated autoantigens were discovered by high-content autoantibody profiling using the bead-based Luminex xMAP platform SeroTag® (Fig. 1).
- In large-scale “omics”-type autoantibody (AAB) profiling studies, autoantibody reactivity of SSc samples was compared to n=343 healthy controls and n=100 SLE samples.
- Novel AAB targets with p-value <0.05 (Mann-Whitney-U-test) and frequency >15% were identified in SSc patients by comparing (dcSSc: n=32, lcSSc: n=50, and SSc overlap: n=9) with controls.
- The mean modified Rodnan skin score (MRSS), mean disease duration (month), and mean age (years) of the SSc cohort was 10.51, 162.5 and 56.94, respectively.

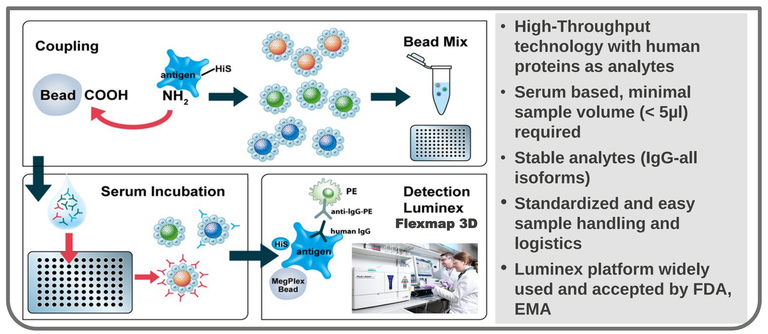

Fig. 1: Schematic SeroTag workflow for the discovery and validation of autoantibodies

Results

NavigAID SSc Assay

A Luminex bead-based AAB assay was designed by combining eight connective tissue disease and two SSc-specific antigens with 12 novel antigens including BICD2, JMJD3/KDM6B, and PPP1R2 (Fig 2). Clinical associations of anti-BICD2 in 502 samples from SSc patients enrolled in the Canadian Scleroderma Research Group (CSRG) cohort will be presented at EULAR 2017 poster SAT0372.

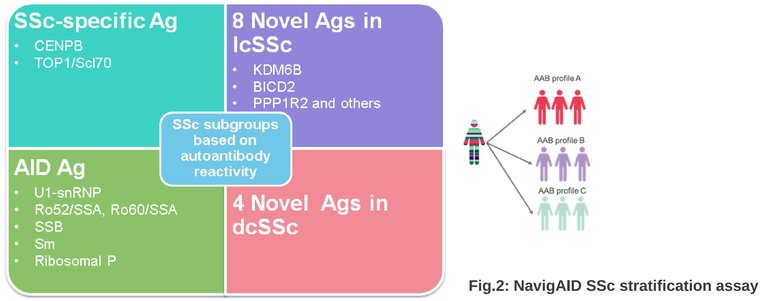

Fig.2: NavigAID SSc stratification assay

To analyze the inter-patient similarity of AAB reactivity, the total number of AABs reactive in each patient was calculated and referenced to the number of all available antigens in percent. Hierarchical cluster analysis of marker co-prevalence and patient signature overlap was performed (Fig. 3).

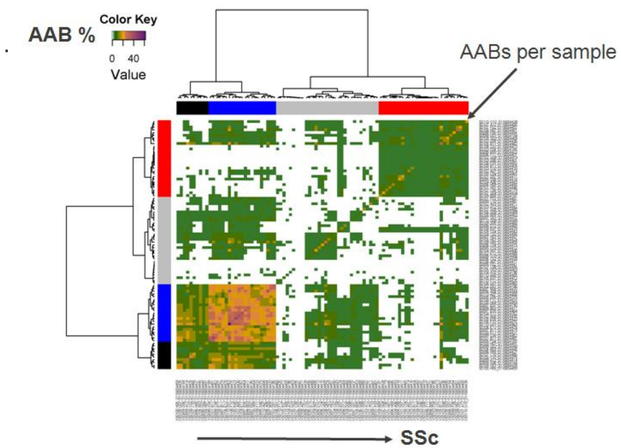

Fig.3: Autoantibody signature similarity and dissimilarity of SSc patients

Autoantibody Reactivity Signatures

Based on their AAB reactivity pattern, the SSc sample cohort can be decomposed into four main clusters:

- Cluster blue (n=21): 90% of all samples were lcSSc, characterized by an extended AAB repertoire (including CENPB, BICD2, KDM6B and PPP1R2), MRSS below the average and longer disease duration.
- Cluster black (n=10): 70% were lcSSc, 20% dcSSc, and 10% SSc-overlap characterized by MRSS below the average. 40% of patients were PPP1R2 positive
- Cluster grey (n=32): 53% were lcSSc, 28% dcSSc, and 19% SSc-overlap. 34% of patients had MRSS below the average and few AABs.
- Cluster red (n=28): 71% were dcSSc, 35% lcSSc, and 4% SSc-overlap. 86% of patients had an MRSS above the average and all had Scl70 AAB.

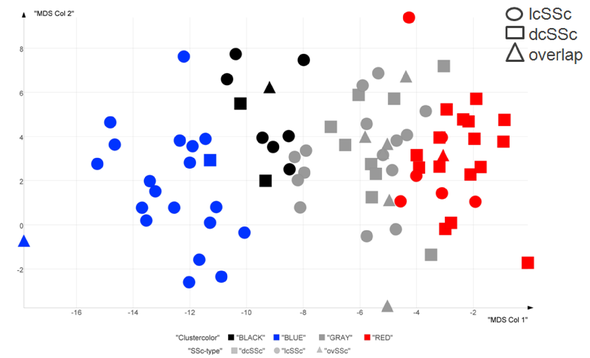

Fig. 4: Interpretation of clusters by Multi-Dimensional Scaling (MDS) analysis

Fig 4 shows a Multi-Dimensional Scaling (MDS) plot of all patients. Patients are labelled according to their cluster membership in Fig. 3.

Conclusions

The multiplexed analysis of AABs in SSc enables defining an AAB reactivity score and SSc patient clusters. This might support the stratification of SSc patients into more homogeneous subgroups in clinical studies thereby increasing the probability of successful drug development.