Introduction

Diagnostic of systemic lupus erythematosus (SLE) is based on a combination of clinical findings and laboratory evidence such as anti-nuclear autoantibodies (ANA), anti-Smith and anti-double stranded DNA (anti-dsDNA) antibodies. However, no biomarker individually displays sufficient performance to diagnose SLE, to predict the disease course or to allow the identification of patient sub-groups. This lack of specific biomarkers also affects clinical development of new SLE therapeutics.

Luminex bead-based antigen arrays were employed to characterize in-depth the autoantibody reactivity of SLE as a source to develop improved diagnostic and patient stratification tests for SLE.

Methodology

The SeroTag® technology enables the discovery and validation of novel autoantigens using an automated multiplex platform (Fig. 2). The SeroTag® technology utilizes the bead-based Luminex xMAP technology which enables to measure the reactivity of autoantibodies to thousands of different antigens in one single serum sample. A crucial component of the discovery process is the unique warehouse of currently 6,500 human proteins expressed in E.coli (1). The NIH-NTA purified proteins are coupled to color-coded magnetic beads which enables the multiplex analysis of up to 500 different antigens. In this study SeroTag® was utilized in a non-hypothesis driven approach to identify novel SLE autoantigens.

Conclusions

- Comprehensive profiling of SLE sera enabled the in-depth characterization of the autoantigen repertoire of SLE patients.
- The combination of established and new antigens significantly increased the sensitivity to diagnose SLE.
- Based on their autoantigen profile SLE sub-groups were revealed.
- Further studies are needed to link specific antigen clusters to clinical response profiles.

Results

Antigen Selection Process

Statistical analysis was performed to distinguish SLE from HV and AIID. Antibodies with at least two-fold difference between the test groups (log2 ratio ≥ 1) and adjusted p-value < 0.05 (log10(1.3)) were selected. Fig. 4 shows the Venn plots in which the magnitude of the antigen reactivity in SLE sera relative to the control group is shown on the y-axis and the statistical significance on the x-axis.

Performance of Antigen Panels

Sequential addition and different combinations of antigens to a panel of known SLE antigens resulted in a stepwise improvement of the classification performance (Fig. 6).

Antigen Clusters and Patient Subgroups

The ability of new antigens to segregate SLE from healthy controls was visualized using Powered Partial Least Squares discriminant analysis (PPLS-DA). The overlaid score (samples) and loading plots (antigens) show that the addition of new antigens significantly improves the separation of SLE samples from HV and reveals further sub-groups of SLE patients.

References