Differential Diagnosis of Autoimmune Diseases, Outlier Detection plus Subgrouping in Clinical Trials by High Content Autoantibody Profiling

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Introduction
Early diagnosis as well as initiation of successful treatment are two big challenges in the management of patients with autoimmune diseases (AID). Overlap of a plethora of clinical symptoms, ranging from multi-organ involvement, fatigue, inflammation to CNS-involvement make differential diagnosis quite challenging. Especially in early disease these signs are difficult to quantify, hence the time from start of disease until clinical diagnosis may be delayed, sometimes for years. With the growing interest in conducting clinical trials in AID, there is a need for new biomarkers that can be used to diagnose individual AIDs to reduce the inclusion of patients not carrying the intended disease, and identify clinical subtypes, predict treatment outcome and assess disease activity.

Objectives
To perform differential diagnosis of AID, to allow for outlier detection and disease subgrouping in clinical trials using our new screening platform SeroTag for quantitative multiplex analysis of known and novel autoantibodies (AAB) in AID.

Methods
The autoantibody reactivity pattern in serum of AID patients was analyzed using a Luminex bead-based antigen array (SeroTag) and 1,600 – 8,000 selected human antigen antigens. We screened over 5,000 serum samples from SjS (n >400), SLE (n >1,800), SSc (n >500), RA (n >2,000), and several other AIDs and over 1,000 healthy individuals to confirm known and to discover novel AABs, and to create reduced autoantibody panels as NavigAID sets for differential diagnosis and disease subgrouping. RUO, LDT and CE- marked tests have been developed as single- and multiplex ELISA (Multilisa).

Results
Identification of Novel Autoantibodies
Apart from clear confirmation of benchmark autoantigens known for many years we have discovered over 80 novel AABs at the SeroTag stage, with frequencies of 10 to >25% in selected AIDs. Some novel AABs are specific for certain diseases, such as the major vault protein MVP in SLE or KDMEB in SSc. Others are present in several diseases, indicating overlap syndromes. Combining novel with known AABs leads to improved panels.

NavigAID Clinical Trial Support
Multiplex panels of 50-100 AABs were generated at the NavigAID stage and applied in over 20 studies. Correlation to laboratory and clinical data at baseline and after treatment (anti-TNF, anti-cytokine/receptor, anti-BAFF) was performed to allow for subgrouping for subsets of diseases according to disease activity, outlier detection, definition of SjS and SLE, and for clear segregation of SjS/SLE overlap syndrome patients. As well, subgrouping of SSc and early RA patients was achieved.

Autoantibody Reactivity Signatures
Self-organizing Maps (SOM) demonstrate the utility of AAB panels to outline an autoimmune landscape, differentiating not only different AIDs but also for subgroup formation within diseases.

Methods
Kohonen maps (SOM) produce a low-dimensional representation of the input space of the training samples called a map. Similar samples are placed next to each other based on 27 autoantibodies. NavigAID.

Conclusions
Sets of 30-90 human protein antigens, half of them well established, the other half novel, succeed in differential diagnosis of AID, in some diseases already at early disease stage. Panels have been used in several drug trials to disect SLE, SjS, or RA into subgroups. Especially in SLE, outliers (10-15% of the trial population) were seen with the option to curate a trial population, eventually to arrive at a more precise assessment of trials primary objectives.

Fig. 1: SeroTag workflow using bead-based arrays.

Fig. 2: NavigAID antigen setup usually combining known with novel antigens. Current extension of work in the immune-oncology space lead to extension of the antigen set.

Fig. 3: MVP has a specific reactivity for SLE.

Fig. 4: Multiplex panel with improved sensitivity for differential diagnosis.

Fig. 5: NavigAID antigen setup usually combining known with novel antigens. Current extension of work in the immune-oncology space lead to extension of the antigen set.

Fig. 6: Integration of clinical trial data (laboratory and outcome parameters) is followed by statistical testing (basic analysis and machine learning procedures).

Fig. 7: Kohonen map analysis of AID patients based on 27 selected AAB-reactivities. The different serum donor groups are healthy controls (blue, red, yellow), SLE (light blue), SSc (pink), SjS (orange), RA (green).

Fig. 8: Disease subgrouping based on AAB-reactivities. A) SjS presents out 5 subgroups, B) Outlier detection in SLE study linked with center effects C) SjS represents as 2 main groups plus 2 intermediate phenotypes.