

VALIDATION OF AN AUTOANTIBODY SIGNATURE FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS IN AN INDEPENDENT COHORT

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease typically characterized by chronic inflammation, accumulation of self-reactive B-cells and production of autoantibodies of which anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor (RF) have diagnostic utility. However, 30% of RA patients remain sero-negative making the early diagnosis of RA more difficult.

In order to characterize the autoantibody repertoire in patients with RA, we performed in an earlier study a large-scale screen against 3,068 antigens using the bead-based Luminex xMAP technology. The autoantibody signature of 74 patients with an established RA was compared against 71 healthy controls. Antigens with high reactivity were selected and used to develop biomarker panels with improved sensitivity and specificity. Having defined this way the autoantibody repertoire of RA patients the goal of this study was to validate the autoantibody reactivity of RA patient samples analyzing 116 pre-treatment samples of early RA derived from the HITHARD treatment study (1).

Conclusions

CCP-negative RA patients can be identified based on a novel, specific set of auto-antibodies. The performance of this marker panels could be verified in an independent cohort of early RA-patients.

Material and Methods

The SeroTag® technology provides a technology platform for the discovery and validation of novel autoantigens using an automated multiplex platform (Fig. 1). 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. The Ni-NTA purified proteins are coupled to color-coded 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 RA autoantigens and to validate the identified candidate antigens in an independent cohort of 116 pre-treatment samples.

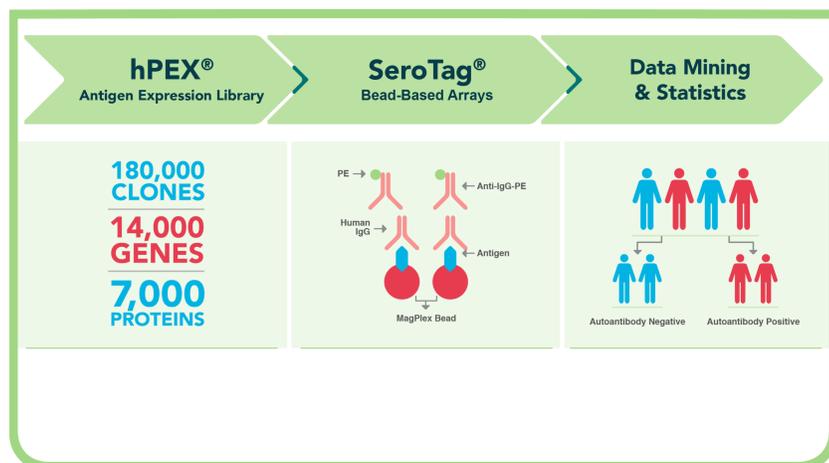


Fig. 1: SeroTag® Process

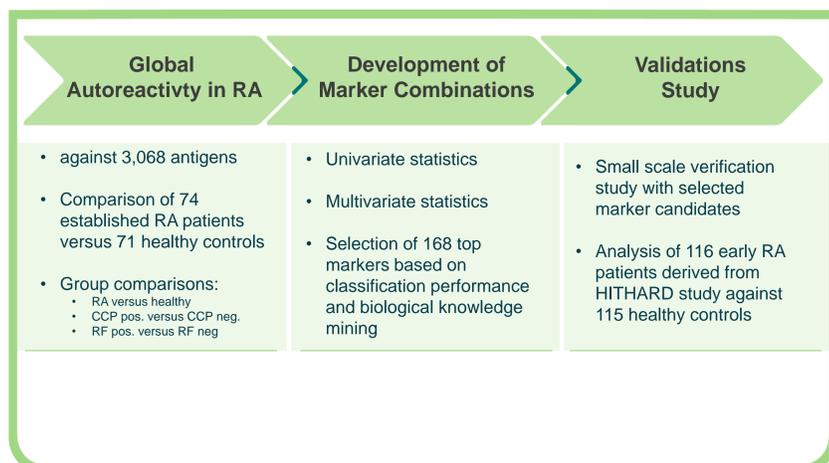


Fig. 2: Discovery and Validation testing

Results

Selection of Antigen Panels

Statistical analysis was performed to distinguish RA from healthy controls including subgroup analysis with respect to CCP and RF status. The best performing single markers are visualized using Vulcano plots (Fig. 3). The magnitude of the antigen reactivity in RA sera relative to the control group is shown on the x-axis and the statistical significance on the y-axis. A citrullinated peptide (citPep) of the first generation (2) as internal standard can be confirmed with the RA cohort.

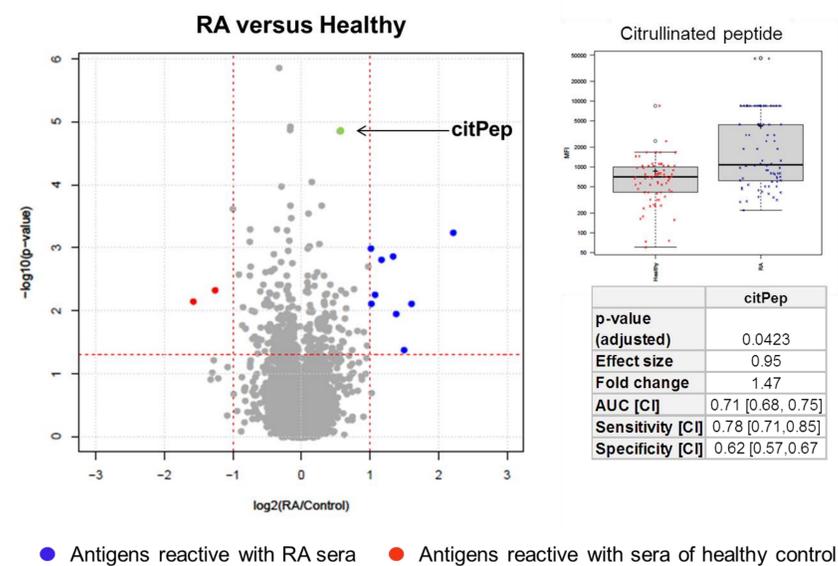
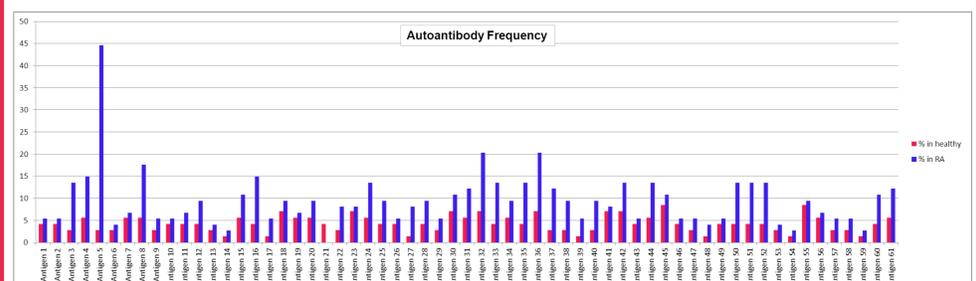


Fig. 3: Visualization of antigen reactivity using fold change and p-value of RA and healthy control cohorts

For biomarker prioritization ranking lists derived from univariate exploratory testing, subgroup analysis (for example: CCP negative versus healthy) and multivariate classification (PPLS-DA and random forest, Top 30 each) were merged. 61 antigens were detected with higher frequency in RA patients than in healthy control (Fig. 4). citPep demonstrated the highest frequency (antigen 5).



For validation the putative candidate antigens were analyzed based on a second RA cohort of 116 RA pre-treatment derived from the HITHARD treatment study. The classification performance of the combination of panel +citPep compared to citPep alone was improved (Fig. 5). This indicates that new marker have been found that have the potential to identify currently seronegative RA patients.

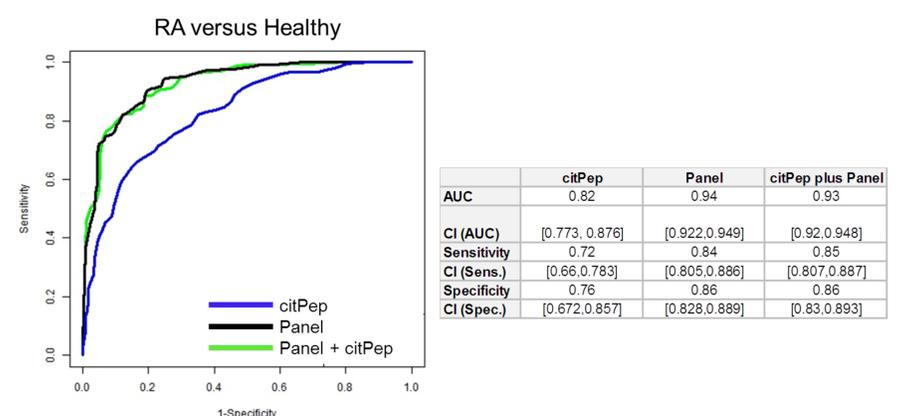


Fig. 5: Classification performance of CCP, new panel and combination of both

References

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- Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. Arthritis Rheum 2000;43(1):155-63.