# Diagnostic Autoantibody Signatures of Rheumatoid Arthritis Patients Identified with a Bead-Based Assay Approach

Angelika Lueking[1], Petra Budde[1], Stefan Vordenbäumen[2], Carmen Theek[1], Heike Goehler[1], Martin Gamer[1], Peter Schulz-Knappe[1], Gerd Burmester[3], and Matthias Schneider[2]
[1]Diagnostics, Protagen AG, Dortmund, [2]Rheumatology, Heinrich-Heine-University Düsseldorf, [3]Humboldt-Universität zu Berlin, Charité – Universitätsmedizin Berlin, Germany

ACR2013-909

## 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.

The goal of this study is to characterize in-depth the autoantibody reactivity of RA patient samples as a source to develop and improve autoantibody-based diagnostic tests.

In order to characterize the autoantibody repertoire in patients with RA, we performed a large-scale screen against 3,068 antigens using the bead-based Luminex xMAP technology. The autoantibody signature of 75 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.

Selected marker candidates were verified analyzing 116 samples of early RA patients derived from the HITHARD treatment study (1).

## Conclusions

- Using the SeroTag® process the profiling of RA sera enabled the in-depth characterization of the autoantigen repertoire of RA patients.
- Multiple antigen/autoantibody interactions exist in serum samples of RA patients with significant differences in comparison to healthy controls. These antigens are candidates for the development of novel diagnostic tests.
- Combining a citrullinated peptide with additional antigens improved the classification of RA patients.
- Further studies are needed to validate the marker candidates with respect to related diseases such as osteoarthritis, psoriatic arthritis, fibromyalgia, …

## References

1. Detert J, Bastian H, Listing J, Weiß A, Wassenberg S, Liebhaber A, et al. Ann Rheum Dis. 2013, 2013;72(6):844-50
2. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. Arthritis Rheum 2000;43(1):155-63.

### Abbreviations

PPLS-DA: powered partial least squares discriminant analysis; AUC: area under the curve; S.D.: standard deviation;

## Methodology

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.

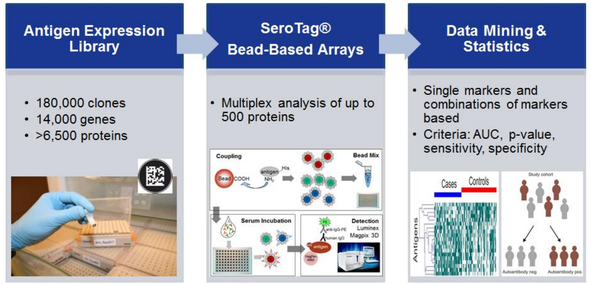



**Fig.1: SeroTag® Process**

## Study Design

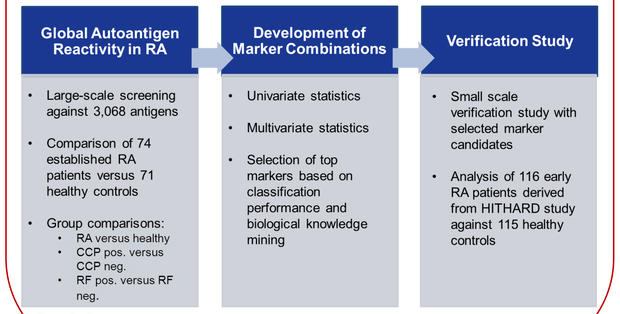
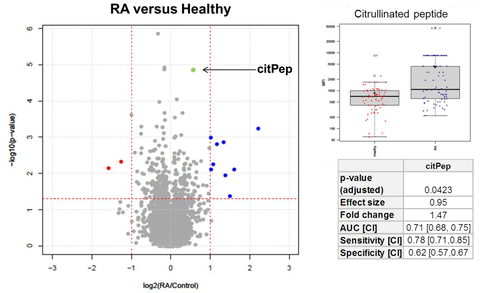



**Fig. 2: Discovery and performance testing**

## 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.




| | citPep |
|---|---|
| p-value (adjusted) | 0.0423 |
| Effect size | 0.95 |
| Fold change | 1.47 |
| AUC [CI] | 0.71 [0.68, 0.75] |
| Sensitivity [CI] | 0.78 [0.71,0.85] |
| Specificity [CI] | 0.62 [0.57,0.67 |

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

For biomarker prioritisation 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. 99 Antigens were considered as relevant as they fulfilled two or more of the criteria:

- p-value <0.05 (especially within the subgroup CCP negative)
- AUC >0.70
- elevated fold change (>1.5)
- Relevant performance within multivariate analyses

A cut-off (mean value plus 2*S.D. of healthy control; citPep: mean value+ 1*S.D. of healthy control) was defined and the frequency of antigens in RA patients determined. 61 antigens were detected with higher frequency in RA patients than in healthy control (Fig. 4). citPep demonstrated the highest frequency (antigen 5).

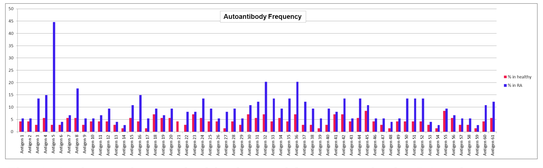
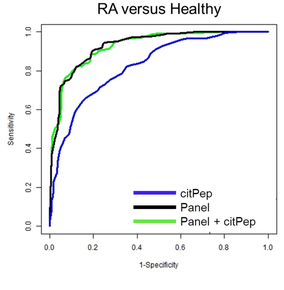



**Fig. 4: Antigens detected with elevated frequency in the RA cohort**

Different panels of antigens were determined to achieve best classification accuracy cumulative to CCP by applying two methods: threshold sensitivity and specificity, and AUC. A panel of six antigens was determined.

### Validation of Marker Candidate Antigens

For validation the putative candidate antigens were analyzed based on a second RA cohort 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.




| | citPep | Panel | citPep plus Panel |
|---|---|---|---|
| AUC | 0.82 | 0.94 | 0.93 |
| CI (AUC) | [0.773, 0.876] | [0.922,0.949] | [0.92,0.948] |
| Sensitivity | 0.72 | 0.84 | 0.85 |
| CI (Sens.) | [0.66,0.783] | [0.805,0.886] | [0.807,0.887] |
| Specificity | 0.76 | 0.86 | 0.86 |
| CI (Spec.) | [0.672,0.857] | [0.828,0.889] | [0.83,0.893] |

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